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BULLETIN

OF THE NEW YORK ACADEMY OF MEDICINE

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H. R. G. DEICHER
and H. G. KUNKEL

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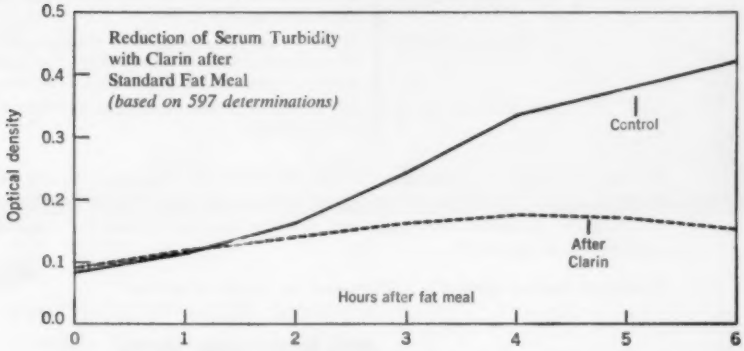

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1. Council on Drugs, J.A.M.A. *166*:52 (Jan. 4) 1958. 2. Hahn, P. F.: Science *98*:19 (July 2) 1943. 3. Fuller, H. L.: Angiology 9:311 (Oct.) 1958. 4. Rubio, F. A., Jr.: Personal communication. 5. Engelberg, H., *et al.*: Circulation *13*:489 (April) 1956.

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1. McClendon, S.J.: Arch. Pediat. *75*:101 (March) 1958.

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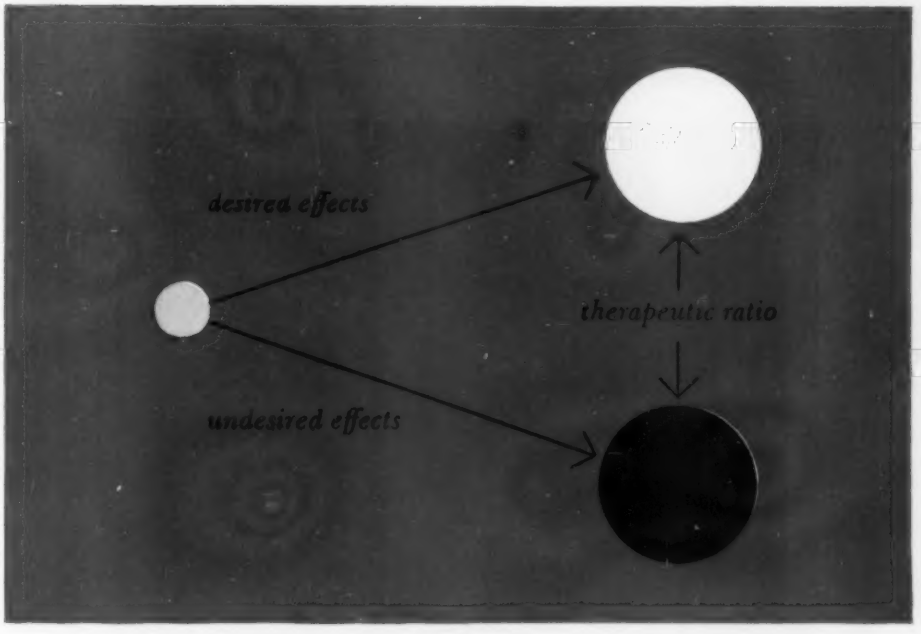

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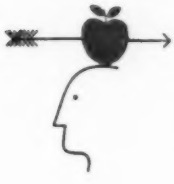

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1. Neustadt, D. H.: Corticosteroid Therapy in Rheumatoid Arthritis: Comparative Study of Effects of Prednisone and Prednisolone, Methylprednisolone, Triamcinolone and Dexamethasone, J.A.M.A., in press.

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REFERENCES: 1. Gearren, J.B.: Dis. Nerv. System *20*:66 (Feb.) 1959. 2. Margolis, E.J., et al.: Scientific Exhibit at 12th Clinical Meeting of the American Medical Association, Minneapolis, Dec. 2-5, 1958. 3. Phillips, F.J., and Shoemaker, D.M.: *ibid.* 4. Ayd, F.J., Jr.: Clin. Med. 6:387 (Mar.) 1959. 5. Tedeschi, D.H., et al.: in *Trifluoperazine: Clinical and Pharmacological Aspects*, Philadelphia, Lea & Febiger, 1958, pp. 23-73.

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*Carter, S.: *M. Clin. North America* 315 (March) 1953.

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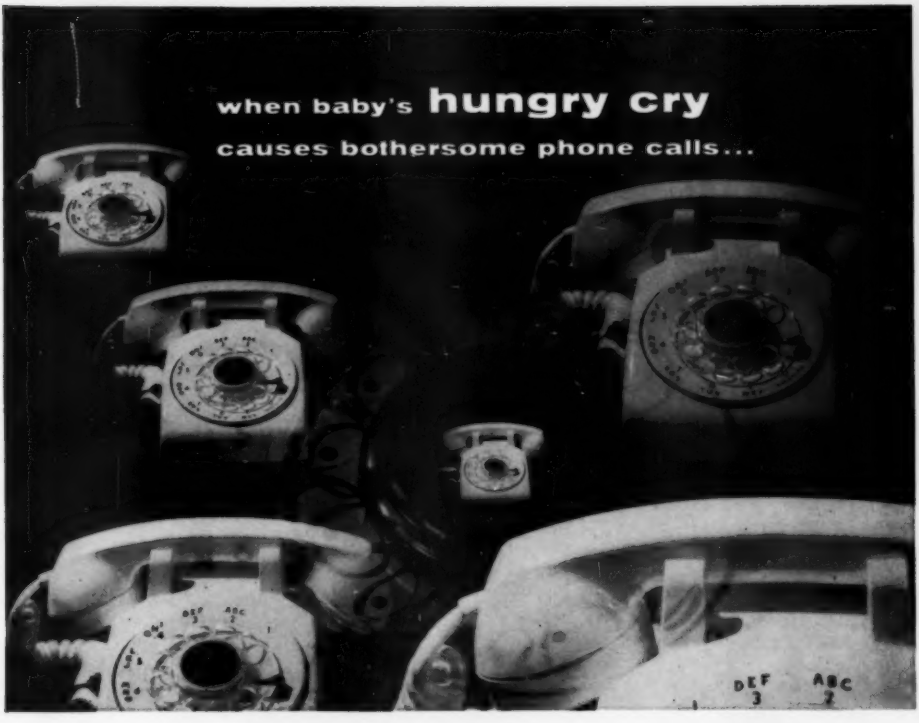

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1. Hatfield, M. A., Simpson, W. A., and Jackson, W. L.: J. Pediat. 44: 32-45 (Jan.) 1954. 2. Frost, L. W., and Jackson, W. L.: J. Pediat. 39: 585-592 (Nov.) 1951. 3. Henrickson, W. E.: GP 8: 51-56 (Oct.) 1953. 4. Litchfield, H. R.: Arch. Pediat. 61: 617 (Dec.) 1944.

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ROBERT L. CRAIG, *Editor*

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BULLETIN OF
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OF MEDICINE

VOL. 35, NO. 7 JULY 1959

THE L.E. CELL AND THE L.E. SERUM FACTORS*

H. R. HOLMAN, H. R. G. DEICHER and H. G. KUNKEL

WHEN Hargraves[1] described the formation of the L.E. cell in the blood of patients with systemic lupus erythematosus (L.E.), he described a biological phenomenon of considerable interest. Here was a serum substance capable of inducing a specific morphologic change in cells of the patient. Clarification of the nature of this phenomenon promised to provide clues to understanding the disease.

Soon thereafter Haserick[2] showed that the serum factor responsible for this morphological reaction migrates on electrophoresis with the γ-globulin. Subsequent observations of the formation of L.E. cells employing both standard staining techniques[3] and phase microscopy[4] have indicated the stages involved in the formation of L.E. cells. Initially, the factor present in the patient's serum acts upon the nucleus of certain white cells and causes visible swelling. This swollen nucleus is then extruded into the surrounding medium and is phagocytized by another viable white cell. This latter white cell, with the swollen nuclear mass within its cytoplasm as a homogeneous basophilic inclusion body, con-

* Presented by Dr. Kunkel at the 2nd Annual Postgraduate Week on *Research Contributions to Clinical Practice*, of The New York Academy of Medicine, October 13, 1958.

From the Rockefeller Institute, New York, N. Y.

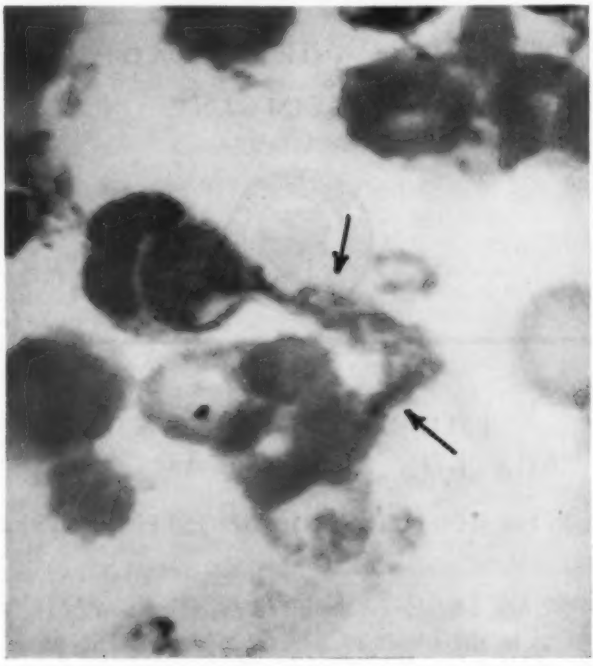

Figure 1.—Phagocytosis of nucleoprotein coated with L.E. cell factor. Nucleoprotein strands used to absorb L.E. serum, then washed and incubated at 37°C. with viable white cells. Arrows show nucleoprotein strand, one end of which has been engulfed by phagocyte forming inclusion body similar to that of L.E. cell. After Wright stain, nucleoprotein has light purple color.

stitutes the L.E. cell. Many methods have been devised to detect L.E. cell formation; all must include three ingredients, the serum factor, a source of cell nuclei to be acted upon by the factor, and viable white cells to phagocytize the affected nuclei.

Initial investigations of the L.E. cell by histochemical techniques[5] led to the view that the serum factor caused depolymerization of the nuclear DNA, presumably by activation of an intracellular DNase. This view now has been altered. Godman[6], also utilizing histochemical techniques, has presented evidence that depolymerization of the nuclear DNA does not occur. Rather, he has demonstrated a striking increase

in the protein content of the affected nucleus. Nuclear swelling and alteration of the staining characteristics accompany this rise in intranuclear protein. Godman's studies also suggested that the protein combines directly with the nuclear DNA.

Examination of the L.E. cell phenomenon by methods other than those of histochemistry was given initial impetus by Miescher[7] in Switzerland. He observed that passage of isolated cell nuclei through L.E. serum left the serum incapable of inducing L.E. cell formation. Subsequent studies[8-12] confirmed his observations and demonstrated the presence of γ-globulin adherent to the nuclei after absorption of L.E. serum. Such nuclei, with L.E. γ-globulin adherent to them, are rapidly phagocytized by normal white cells with the formation of inclusion bodies indistinguishable from those of L.E. cells. If the DNA is removed from the nuclei by digestion with DNase the nuclei can no longer absorb the L.E. cell-inducing factor. Whole nuclei are not necessary for absorption of the factor, however; isolated nuclear nucleoprotein may be substituted. When nucleoprotein is passed through L.E. serum, it absorbs quantitatively the L.E. cell-inducing factor. Gammaglobulin can then be shown to be adherent to the nucleoprotein strands and these strands are phagocytized by viable white cells, which then resemble L.E. cells (Figure 1). These results indicate that the L.E. cell factor acts by directly combining with the cell nucleus, most likely the nuclear nucleoprotein. The staining characteristics of the nucleus are altered as a result, and the nucleus becomes subject to subsequent phagocytosis. Nuclear DNA appears necessary for this interaction.

Exploration of the reaction by complement fixation techniques[13, 14] shows that L.E. serum can fix complement not only with cell nuclei and nucleoprotein, but also with other constituents of the cell nucleus. Many L.E. sera will react with a material extractable from cell nuclei with low ionic strength buffers, and some sera will react with isolated DNA or histone. The reactive substance in the buffer extract appears to be neither nucleic acid nor histone, for it is resistant to both nuclease and trypsin digestion. Examples of complement fixation reactivity may be seen in Table I. Individual sera possess different titers of reactivity against the various constituents of the nucleus, and a given serum may react only with some of the various nuclear constituents. In general, the ability to fix complement with whole nuclei, nucleoprotein and the buffer extract of nuclei is much more common than the ability to fix

TABLE I

Serum	*L.E. Cell formation*	*Antigen used in C.F.*	*Serum Dilution* 2	4	8	16	32	64	128	256	512	1024
Tr.	strong	Nuclei	4	4	4	4	4	4	4	4	2	0
		PO4 Extract	4	4	4	4	4	4	4	4	4	0
		Nucleoprotein	4	4	4	4	4	4	4	4	2	0
		DNA	4	4	4	3	0	0	0	0	0	0
		Histone	0	0	0	0	0	0	0	0	0	0
Fr.	strong	Nuclei	4	4	4	4	4	4	1	0	0	0
		PO4 Extract	4	4	4	4	4	4	4	4	0	0
		Nucleoprotein	4	4	4	4	4	4	4	2	0	0
		DNA	0	0	0	0	0	0	0	0	0	0
		Histone	4	4	4	4	0	0	0	0	0	0
Me.	strong	Nuclei	4	4	4	0	0	0	0	0	0	0
		PO4 Extract	4	4	4	4	4	0	0	0	0	0
		Nucleoprotein	4	4	4	4	4	4	0	0	0	0
		DNA	4	4	4	4	2	0	0	0	0	0
		Histone	0	0	0	0	0	0	0	0	0	0

Complement fixation between lupus erythematosus serum and constituents of the cell nucleus. Serum dilution method. Two 100 per cent units of complement employed. 4 denotes complete fixation. 0 denotes complete hemolysis.

complement with DNA or histone alone. Histone fixation is rare. These results, plus those from cross-absorption studies, suggest that L.E. serum may contain a number of different factors capable of reacting with different components of the cell nucleus.

The identity of the nuclear constituent directly involved in the formation of L.E. cells has been clarified further by exploration of the reaction between the L.E. cell factor and nucleoprotein. Prior to this exploration, a means of recovering the L.E. cell factor from nucleoprotein was sought so that the interaction could be studied not only by absorption of the factor but also by its recovery from the absorbing material. Once absorbed by nucleoprotein, the L.E. cell factor is not readily liberated. It is not removed by elution at higher temperatures or at pH's below 8.6. Elution at pH's above 8.6 or in higher concentrations of salt cannot be employed because the nucleoprotein dissolves. However, digestion of the nucleoprotein-L.E. cell factor complex with DNase provided a solution. When the complex is digested with

TABLE II

Type of Nucleoprotein	*Normal*	*DNA removed*	*Histone removed*	*Reconstituted after histone removal*	*"Synthetic" DNA-Histone*	*"Synthetic DNA-Protamine*	*DNA alone*
L.E. cell formation by serum after absorption	—	+++	+++	++	++	++++	++++
Recovered L.E. cell factor from nucleoprotein	++++	+	+	++	++	—	—

Absorption of L.E. cell factor by nucleoprotein extracted from nuclei and altered in the indicated manner, or prepared in the laboratory by combination of purified constituents.

See text for discussion.

DNase, DNA and γ-globulin are liberated. This γ-globulin has negligible ability to induce L.E. cell formation. It can, however, fix complement with nuclei, nucleoprotein, and DNA. After nuclease digestion there remains an insoluble residue consisting of the bulk of the original histone plus a considerable amount of γ-globulin. Much, but not all, of this latter γ-globulin can be liberated by eluting the residue at 56° in isotonic saline or at 37° with 2 N saline. This γ-globulin is capable of inducing L.E. cell formation to approximately the same extent of the original serum, and it fixes complement with cell nuclei and nucleoprotein but not with DNA. Thus it is possible to recover the L.E. cell factor from a complex with nucleoprotein by a two-stage procedure; the first is digestion of the complex with DNase, and the second is elution from the insoluble residue at higher temperature or higher salt concentrations.

If the DNA is removed from nucleoprotein by DNase digestion, or the histone is removed by extraction with 0.1 N hydrochloric acid, insoluble residues are left. The first of these contains approximately 80 per cent of the original histone and non-histone protein, and the second contains 100 per cent of the original DNA. Absorption of the L.E. serum with either of these residues results in removal of only a minimum amount of L.E. cell-inducing factor. Furthermore, after such absorption, only small quantities of L.E. factor could be recovered from the ab-

Figure 2.—The precipitin reaction between DNA and L.E. serum in a double diffusion agar precipitin tube. Tube on left shows precipitin band and contains L.E. serum as lower layer, 0.5 per cent agar as middle layer, and a solution of DNA, 100 γ per ml., as upper layer. Tube on right contains normal serum. For details see reference 15.

sorbing material. The reconstitution of nucleoprotein by recombining the extracted histone with the residual DNA, or by adding DNA to the residual histone, yields materials which resemble nucleoprotein but which are generally incapable of absorbing L.E. cell factor, though some modest reconstitution is attained when the extracted histone is recombined with the residual DNA.

A "synthetic" nucleoprotein can be made by combining isolated DNA with isolated histone. The product physically resembles extracted nucleoprotein, but is much less capable of absorbing L.E. cell factor than extracted nucleoprotein when they are compared on a weight basis. Isolated DNA is incapable of absorbing the L.E. cell factor. These results are summarized in Table II. It appears, therefore, that the L.E. cell factor reacts directly with nuclear nucleoprotein and requires both the DNA and the histone of the nuceloprotein for its combination.

The L.E. cell-inducing factor requires DNA for its reaction but does not appear to react with DNA alone. However, complement fixation studies indicated that certain L.E. sera can react directly with DNA. This reaction with pure DNA has been explored in our laboratory[15] and by Seligmann[16], Ceppellini[17], and Miescher[18] in Europe. Reactive sera will form precipitin bands with DNA in agar diffusion tubes (Figure 2) and, under conditions conducive to quantitative precipitin reactions, will yield a precipitin curve with apparent equivalence and

antigen excess zones. These reactions occur between pH 7 and pH 8.6 where other sera do not show a precipitin reaction with DNA, and where the nonspecific interaction between DNA and serum proteins has not been found to occur. DNase digestion of the precipitate which occurs at equivalence point in this system liberates γ-globulin. This γ-globulin can fix complement with DNA and will reprecipitate if fresh DNA is added. Serum from which the DNA precipitate has been obtained at equivalence point still retains its capacity to induce L.E. cell formation. The γ-globulin which is recovered by DNase digestion of the DNA precipitate has been found to possess little or no L.E. cell-inducing capacity.

Thus L.E. serum may contain at least two factors which react with DNA. One of these is the L.E. cell factor and requires not only DNA but also the protein of the nucleoprotein for its reaction. The other factor, present less frequently, can react with DNA alone and does not appear to possess significant ability to form L.E. cells.

The recovery of fairly pure L.E. cell factor and DNA precipitating factor from their respective combinations with nucleoprotein and DNA has permitted study of their properties. Both of these factors migrate electrophoretically with γ-globulin and sediment ultracentrifugally with the bulk of human γ-globulin. The L.E. cell factor is quantitatively precipitated and completely inactivated by antiserum to normal human γ-globulin. The L.E. cell factor and the DNA precipitating factor form single bands in agar diffusion against antiserum to normal human γ-globulin and to whole normal human serum. The reaction between the DNA precipitator and the DNA resembles a classical precipitin reaction. Though the serum factors which react with other nuclear constituents have not yet been isolated, they too migrate with γ-globulin electrophoretically and sediment with γ-globulin ultracentrifugally. These characteristics suggest that the multiple antinuclear factors which appear in L.E. serum may be a family of antinuclear antibodies. Furthermore, since they are capable of reacting with the patient's own cellular material, they may be auto-antibodies.

However, although the characteristics which have been identified are compatible with an antibody nature of these factors, proof of such a nature is not complete. Particularly lacking is evidence that analogous factors can be induced in experimental animals which have been im-

munized with nuclear constituents. Such immunization of both normal rabbits and rabbits chronically fed Apresoline has, in our hands, yielded only complement fixation reactions with a constituent of the cell nucleus which is sensitive to trypsin. We have failed to obtain any nucleic acid reacting substance. Factors in animal sera which fix complement with DNA have been reported from other laboratories under certain experimental conditions [19-21], but there is as yet no evidence that these nucleic acid reacting factors are analogous to the serum factors found in systemic lupus erythematosus. The search for experimentally induced antinuclear antibodies may never prove entirely fruitful. If such antibodies develop in the patient because of an abnormal immune response on the part of a patient to a normal body constituent, then it may be impossible to obtain analogous factors in experimental animals until such an abnormal immune response has been induced in the animals.

Patients with systemic L.E. have been the only ones we have found whose serum will react with the nucleic acid containing constituents of the cell nucleus. Patients with L.E. will also react with a homogenate or extract of whole tissues in which the reactive portion appears to be a constituent of the cytoplasm rather than of the nucleus. However, this latter reactivity is also present in sera from patients who clinically have other diseases giving rise to hypergammaglobulinemia; in particular, various types of chronic liver disease and scleroderma. Occasionally patients in this latter category will also show some reaction with whole nuclei, presumably due to the buffer extractable substance in nuclei. However, there has been no reaction with nucleic acid constituents of the nucleus. Complement fixation titers against nuclear constituents in S.L.E. appear to vary with the degree of activity of the disease, being highest during acute episodes and subsiding during spontaneous or therapeutic remission. The L.E. cell factor also shows this behavior.

The relationship of the antinuclear factors to the pathogenesis of the disease or the initiation of any of the pathologic lesions remains to be determined. The evidence to date suggests that these factors do not play a decisive pathogenic role. They are unable to penetrate white blood cells *in vivo* except under unusual circumstances and do not appear to be present on the nuclei of tissues obtained at postmortem examination[22, 23]. In our hands, they have not interfered with the growth of normal or tumor cells in tissue culture. Thus, it appears that they cannot

gain access to the nucleus of a viable cell, and must await some alteration in the permeability of the cell membrane before they can enter the cell and reach the nucleus. At the moment, therefore, they may best be characterized as by-products of an altered immune system. However, exploration of this question is in its infancy.

Our present understanding of the L.E. cell phenomenon holds that it is the result of an interaction between nuclear nucleoprotein and one of a family of antinuclear antibodies present in the serum of the patient, and that this interaction is of an immune nature. If the future validates this hypothesis, then it may be said that Hargrave's discovery has opened a most interesting field for biologic study. The family of antinuclear antibodies, of which the L.E. cell factor is but one, may prove to be the first good evidence of the potential antigenicity of nucleic acids. Study of the conditions governing the appearance of these antibodies to a constituent of the cells of such importance as the nucleus, may assist in our understanding of autoimmunity in general, as well as of the underlying disorders in this disease.

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THE EXPANDING PATTERN OF THE INBORN ERRORS OF METABOLISM*

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In the late 1890's a young English physician, Archibald E. Garrod, encountered his first cases of alkaptonuria, and thereupon embarked upon studies which led him to a grand conception of extraordinary fruitfulness[1, 2], that of inborn errors of metabolism. In his Croonian Lectures of 1908[2] Garrod described the disorders in question as "metabolic sports, the clinical analogues of structural malformations" and gave a startlingly modern account of their origin as genetically transferred deficiencies of one or another enzyme required to perform a specific metabolic function. Grouped together in this common category were alkaptonuria, albinism, cystinuria and pentosuria; and even these few examples were cited more by prescience than by the demonstration of the scanty evidence then available, as Knox[3] has explained so engagingly. The few examples indicated were, however, "merely the obvious members of a far larger group", recognizable only because they "advertise their presence in some conspicuous way". They were to be clearly distinguished on the one hand from general disorders of metabolism, and on the other from the minor metabolic variations which occur within the framework of the normal in different individuals.

The concept was so far in advance of the enzymological techniques necessary to support it that its implications were not fully realized until recent years. Now it is plain that no segment of the total of metabolic processes is immune from inborn metabolic errors. For while such defects immediately involve the structure of proteins (enzymes), the functional incapacity of a critical enzyme may be reflected in profound disturbances in the metabolism of proteins, amino acids, carbohydrates, lipids, nucleic acids, porphyrins, electrolytes or pigments. To illustrate this diversity the following examples of inborn errors of metabolism

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may be cited: hemophilia and the parahemophilias, the hemoglobinopathies, the congenital methemoglobinemias, a variety of hemolytic anemias, the porphyrias, Gilbert's disease, Dubin-Johnson's disease, congenital agammaglobulinemia, a variety of connective tissue disorders, cystinosis, oxalosis, Wilson's disease, phenylketonuria, essential hypercholesterolemia and hyperlipemia, the lipidoses, galactosemia, the glycogen storage diseases, several varieties of sporadic familial cretinism, hypophosphatasia, familial periodic paralysis, a variety of renal tubular reabsorptive defects, some of the progressive muscle dystrophies and myotonias. Many others are known, and more doubtless remain to be uncovered.

Inborn errors of metabolism are presumed to originate by mutation of the chromosomal nucleic acid template from which the apoenzyme is generated, in what appears to be a one gene-one enzyme relationship. It is not clear whether the missing enzyme is in fact wholly absent, or really present but structurally so malformed as to render it more or less functionless. How fine a change in protein structure may suffice to alter its properties significantly was demonstrated by Hunt and Ingram[4] in the case of hemoglobins S and C. At just one point in the long chain of amino acid sequences which constitute the globin molecule, the glutamic acid of hemoglobin A is replaced by valine in hemoglobin S and by lysine in hemoglobin C. The resulting changes in surface charge permit separation from hemoglobin A by electrophoretic procedures, which makes ready diagnosis possible. *In vivo*, the minute defect in the composition of the globin moiety of hemoglobin S appears to be responsible for the characteristic abnormal aggregation of the molecules within the red cells when oxygen is lost (sickling). In the case of hemoglobin C, there appear to be differences also in the position of the free sulfhydryl groups[5]. It is a sobering thought that such infinitesimally small structural malformations at the molecular level may be as lethal as gross abnormalities of vital organs that are patently incompatible with life. Indeed, what we commonly think of as morphological abnormalities may in the final analysis prove to be distortions in the grand plan of development deriving from some accident of molecular arrangement[6]. This is obliquely suggested by the effects of maternal vitamin deficiencies in teratogenesis, indicating that certain vitamins (coenzymes) "play a highly significant role in embryonic differentiation and organogenesis"[7].

Fortunately, relatively few inborn errors of metabolism have dire consequences. Those that "advertise their presence" are of course easily recognized, but most functional deficiencies doubtless cause inapparent over-all changes in the processes of metabolism, and thus escape ready recognition even with modern methodology; Garrod in fact stressed the benign nature of the inborn errors of metabolism known to him[1, 8, 9]. This applies particularly to the partial metabolic blocks of the heterozygote, in whom morphological and even metabolic abnormalities are apt to be so minor as to be detectable, if at all, only by the most refined techniques. It is when a critical metabolic pathway is wholly blocked that serious clinical consequences ensue, and even then adaptive alternate pathways may suffice to take over a substantial part of the burden of the missing enzyme. This may occur, for example, in galactosemia. Here there is a deficiency of galactose-1-phosphate uridyl transferase, an enzyme necessary for the conversion of galactose-1-phosphate to glucose-1-phosphate, and as a result galactose-1-phosphate and galactose accumulate in the blood and tissues. However, even when the transferase is completely absent, there is some conversion of galactose to glucose by an alternative route[10]. Moreover, it is likely that some adaptive processes increase with age, the alternative pathways slowly compensating more fully for the metabolic lack; this would account for the frequency with which children outgrow the more obvious disabilities of some inborn errors of metabolism.

Despite their genetic transmission, inborn errors of metabolism are only exceptionally obvious at birth. The period required for demonstration, if indeed the defect is detectable at all, may be a matter of days or weeks, when dependent upon exhaustion of maternal supplies, the effect of feedings (galactosemia) or the maturation of accessory enzymes (alkaptonuria)[11]; or many years may elapse before the underlying metabolic error is established, as in the first appearance of gouty hyperuricemia at puberty in males, or the even later development of hypercholesterolemia in essential hypercholesterolemia. Clinical deficits, when they occur, are apt to be even longer delayed. It is apparent that expressivity ordinarily is the result of a complex interaction of genetic, metabolic and environmental influences involving the interplay of diet, endocrine functions, intercurrent diseases, the action of drugs, emotional stresses and other factors. In the storage diseases, time is required for accumulations sufficient to cause significant functional disabilities. Com-

plications that are secondary to the primary defect may then dominate the clinical syndrome, as in the cumulative deposition of copper in selective areas of the brain, liver and kidney in Wilson's disease, or the deposition of cystine in the kidney, viscera and bones in cystinosis.

The diversity of the clinical patterns of the inborn errors of metabolism is, in part, a reflection of the diversity of the specialized tissue functions which may preferentially be impaired. Thus hemolytic anemia is the principal clinical deficit if the deficiency involves a component of the erythrocyte enzyme systems required for the utilization of glucose (enolase, it is believed, in hereditary spherocytosis, glucose-6-phosphate dehydrogenase in favism), and there is, consequently, an abnormal susceptibility to conditions favoring hemolysis. Disturbances in bone formation are particularly prominent in hypophosphatasia, a deficiency of non-specific alkaline phosphatase; cretinism is present when an enzyme required for coupling of iodotyrosine is lacking; periodic paralysis when there is a fault in the potassium metabolism of muscle; and similarly in many other systems.

In a special category is a group of hereditary disorders characterized by defective renal tubular reabsorption of one or more constituents of the glomerular filtrate, presumably a reflection of deficient enzyme transport systems resident chiefly in the proximal convolution. The reabsorptive defect may be highly selective (renal hypophosphatemia, glycinuria), confined to a few components (cystinuria), or may include a variety of electrolytes and metabolites (Fanconi syndrome). The increased renal clearance—whether of amino acids, hexoses, pentoses, potassium, phosphate, calcium, bicarbonate, or water—is associated with low or low normal plasma concentrations of the components in question; this is in sharp contrast to the high blood levels, with overflow into the urine, found when there is a metabolic block and accumulation of a metabolite in the tissues (*cf.* cystinuria and cystinosis, for example). The depletion which results from failure of renal conservation may have untoward effects, as in the development of rickets or osteomalacia in renal hypophosphatemia or in cystinosis with the Fanconi syndrome. In some instances the chief hazard is renal calculus formation, when a constituent of low solubility is excreted in the urine in excess, as in cystinuria, oxaluria, idiopathic hypercalcinuria and glycinuria. It is of interest in this connection that similar defects in renal tubular reabsorption occur in hereditary strains of animals, for example, the impaired

tubular reabsorption of uric acid in the Dalmatian coach hound, and of cystine in some Irish terriers. The blotched genet, in fact, is said to have a species-specific defect in tubular reabsorption of cystine, and normally is cystinuric. Such species differences characterized by enzyme deficiencies probably are widespread; witness the absence in normal man of the enzyme necessary to convert L-gulonolactone to ascorbic acid[12], thus making man dependent upon dietary sources for vitamin C.

Another special category is made up of a variety of inborn errors of metabolism which ordinarily are wholly inapparent, the subject giving every evidence of normal health until exposed to a particular stress, which then reveals the innate deficiency. (This eventuality was clearly envisioned by Garrod[8], who spoke of the probable role of inborn errors of metabolism in idiosyncrasies.) The stress may take the form of drug administration, as in the case of untoward reactions to succinylcholine, ascribable to deficient pseudocholinesterase activity. A particularly striking example is deficiency of erythrocyte glucose-6-phosphate dehydrogenase[13], a TPN-dependent enzyme which accelerates the reaction to 6-phosphogluconolactone and TPNH; the latter being required for stability of normal erythrocyte levels of reduced glutathione, and of the intact red cell. Subjects with an inherent deficiency of erythrocyte glucose-6-phosphate dehydrogenase may be apparently normal but peculiarly susceptible to the hemolytic effects of a host of drugs, such as primaquine, naphthalene, sulfanilamide, nitrofurantoin, and to fava beans in the dietary. It is worth noting that by elucidation of this common pathogenesis, drug and dietary idiosyncrasies that would otherwise be completely disparate in etiology are brought together as expressions of a single faulty mechanism. It may be presumed that other drug-hypersensitivity reactions also may derive from one or another enzyme deficiency.

The most characteristic inborn errors of metabolism, however, are those that reflect a block, due to a specific enzyme deficiency, in the long sequence of enzymatic reactions required for biosynthesis or degradation in the various metabolic pathways. The operation of such blocks has been informatively discussed by Dent[14] and Snyder[6]. Sometimes it is lack of the end product of the chain of reactions which is the most conspicuous abnormality of the metabolic error, as in the deficiency of melanin in albinism. More often it is accumulation of the metabolite(s) antecedent to the site of enzymatic block which is apt to

dominate the clinical picture. These may cause difficulties in disposal, as in the storage diseases, due to the sheer bulk of tissue deposits of the metabolite, with displacement of vital tissue elements; or the deposits may elicit a vigorous connective tissue reaction with like results, as in the cirrhosis of hemochromatosis and in gouty tophi and nephropathy. Other more devious ramifications of accumulation of intermediary metabolites depend upon their "toxic" effects, as is suspected of galactose-1-phosphate in cataract formation and other manifestations of galactosemia[10]. Of particular interest in this connection is phenylketonuria[15], an inborn deficiency of phenylalanine hydroxylase. The characteristic fair hair of this disorder is an indication of interference with melanin formation, which has been attributed to secondary tyrosine deficiency and to the inhibitory effect of accumulated phenylalanine on tyrosinase or related enzymes. The mental deficiency has been ascribed, in part, to a secondary deficit of 5-hydroxytryptamine, said to be due to inhibition of 5-hydroxytryptophane decarboxylase, perhaps by phenylpyruvic acid. The characteristic appearance in the urine of phenylpyruvate, phenyllactate, phenylacetate, and other metabolites[15] reflects the operation of alternate pathways for the metabolism of the accumulated phenylalanine.

There may be a common clinical end-result when a long chain of metabolic reactions is interrupted, due to specific inborn enzyme deficiencies, at any one of several points. In this event, what appears to be a nosologic entity is, in fact, a multiple disorder, each clinically undifferentiated disease having a distinct metabolic pathogenesis. A multiple pathogenesis of this kind has been demonstrated for glycogen storage disease of the type described by von Gierke. This may result from any one of three distinct inborn errors, lack of glucose-6-phosphatase, or of "branching" enzyme necessary for normal glycogen synthesis, or of "debranching" enzyme required for glycogen degradation[16], and consequently embraces at least three discrete diseases. The enzymatic defect underlying generalized glycogenosis (the cardiac form of the glycogen storage diseases) does not correspond to that of any of the three indicated, and thus represents a fourth disorder. It has been suggested that multiple disturbances in purine metabolism may be included in the clinical syndrome of what is now designated primary gout[17]. More precise analysis of the pathogenesis of diabetic and other metabolic disorders may disclose many additional examples of this multi-

plication of causes.

Although no way has been found to restore missing intracellular enzymes, substantial progress has been made in circumventing the metabolic distortions caused by many inborn errors of metabolism. When the defect is limited to circulating proteins, as in hemophilia and congenital agammaglobulinemia, replacement therapy has proved entirely feasible. Depletion of circulating electrolytes also is amenable to replacement therapy, as by the administration of potassium in familial periodic paralysis. Iron and erythrocytes may be supplied to supplant losses in the congenital hemolytic anemias. Excessive intestinal absorption of iron in hemochromatosis can be counteracted by periodic bloodletting; excessive copper deposits in the tissues in Wilson's disease reduced by chelating agents such as penicillamine. Uric acid deposits in gout can be mobilized by the use of uricosuric agents. It is possible to minimize renal calculus formation in gout, cystinuria, oxaluria, glycinuria and idiopathic hypercalcinuria by maintaining large urine volumes and an appropriate urinary pH. In some instances it has been possible to reinforce the action of alternate metabolic pathways, as by the administration of methylene blue or ascorbic acid in congenital methemoglobinemia, and by massive vitamin D therapy in renal hypophosphatemia. Decreased glucose-6-phosphate dehydrogenase activity and reduced glutathione content of erythrocytes can be detected before candidates for therapy are exposed to drugs causing hemolysis, thus making it possible to avoid such drug reactions. Prophylactic measures can be instituted early in inborn errors of metabolism like galactosemia, recognizable in inapparent cases by detection of erythrocyte UDP-Gal transferase deficiency or by the galactose tolerance test, and phenylketonuria, recognizable by appropriate testing of the urine. Early institution of special diets deficient in galactose averts the clinical manifestations of galactosemia, and omission of phenylalanine from the diet has been found beneficial in phenylketonuria. Disabilities already established may be reversed to a considerable degree by such dietary restrictions.

Concluding Remarks

Garrod's grand conception, then, has led, in its many ramifications, to a multitude of interesting and important developments. Investigation of the pathogenesis of inborn errors of metabolism has elucidated many

pathways of normal as well as abnormal intermediary metabolism. The biochemistry of genetics has been much enriched. New diseases have been discovered, and the mechanisms of causation of old ones clarified. Moreover, better understanding of these disorders has suggested means of prophylaxis and treatment which in many instances has led to greatly improved management.

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PHENYLKETONURIA AND MAPLE SUGAR URINE DISEASE*

JOSEPH DANCIS**

My presentation will be brief because the message is simple. I would like to review, more or less historically, the progress that has been made in understanding phenylketonuria, and then demonstrate how this has paved the way for unravelling the mysteries of a newly recognized metabolic disease, maple sugar urine disease.

PHENYLKETONURIA

The major clinical manifestations in phenylketonuria are mental retardation, which tends to be familial, and a peculiar odor to the patient. When Fölling demonstrated phenylpyruvic acid in the urine, it became evident that this represented a metabolic aberration. The similarity in structure of the compound to phenylalanine suggested a relation, but the nature of the relation was not clear for several years. A great stride was made when Jervis fed large amounts of phenylalanine to both normal and phenylketonuric subjects[1]. He found an increase in a Millon-reacting substance in normals and not in phenylketonurics. The Millon reaction detects the phenolic group in tyrosine. The observation therefore indicated that phenylketonurics were unable to convert phenylalanine to tyrosine. Further evidence for such a block was provided, when methods became available, by the demonstration of the extreme elevations of serum phenylalanine in the phenylketonuric patient. And recently the evidence has been made complete by the isolation of a liver enzyme by Udenfriend[2] which converts phenylalanine to tyrosine and by the demonstration by Jervis[3] that this function is deficient in the phenylketonuric liver.

Once the block had been demonstrated and localized, the approach to the next important question was given direction. Why did these

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**Markle Scholar in Medical Science.

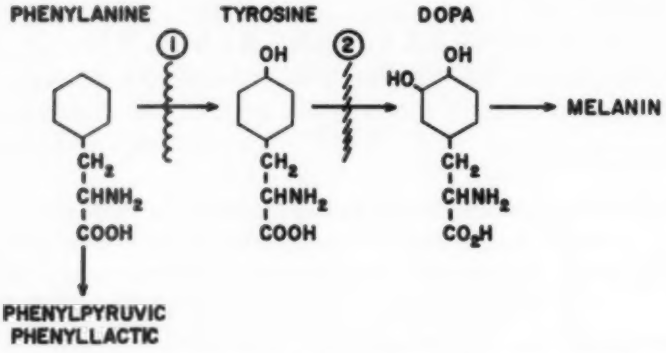

Fig. 1 — Interference in melanin formation in phenylpyruvic oligophrenia.
(1) Metabolic defect—failure to convert phenylalanine to tyrosine.
(2) Point of postulated competition of excess phenylalanine with tyrosine for the enzyme tyrosinase.

patients get into such serious trouble? Was it deficiency of materials beyond the block, or an excess of materials in front of the block (Figure 1)? Two separate and concurrent observations suggested a mechanism. Drs. Holt and Snyderman[4], working on the theory that there might be insufficient tyrosine, fed large amounts of this amino acid to four phenylketonurics. One of the patients had been blonde but now his hair began to darken. It had been an old clinical observation that phenylketonurics tended to be blonde, even though their families were brunette. Now it appeared that this might be because of tyrosine deficiency.

However, others, working on the theory that an excess of phenylalanine or its products might be harmful, had been feeding phenylketonurics a low phenylalanine diet[5]. The hair of these patients also turned dark. Here was a paradox. Certainly, lowering the phenylalanine intake could not of itself raise the tyrosine level. Instead, it suggested that the *relative* levels of the two amino acids were the significant feature. The close similarity in structure of the amino acids suggested that an excess in phenylalanine might compete with the tyrosine that is normally available from the diet for the enzyme, tyrosinase. This enzyme is necessary for formation of dihydroxyphenylalanine, which in turn is converted to melanin (Figure 1).

TABLE I—THE EFFECT OF L & D PHENYLALANINE ON THE TYROSINE-TYROSINASE SYSTEM AS SHOWN IN PIGMENT FORMATION

A.	Control	.880
	L-phenylalanine	.590
B.	Control	.830
	D-phenylalanine	.840

Pigment formation indicated as optical density read at 700 mu.

This possibility was tested in the laboratory[6]. Tyrosine was incubated with tyrosinase and the formation of pigment measured in the spectrophotometer. In some flasks L-phenylalanine was also added. As a control, the effects of the unnatural isomer, D-phenylalanine, was also measured (Table I). There was a reduction in pigment in the tubes containing L-phenylalanine and none with the stereo-isomer.

Translating the results of an *in vitro* experiment to the patient is always precarious. In this instance the experiments were designed to test a hypothesis based on clinical observations. The results support the thesis that an excess of phenylalanine can interfere with pigment formation and that this may be corrected by either lowering the phenylalanine or raising the tyrosine. Of course, being blonde or brunette is of little importance to a phenylketonuric. The significance of the experiment is that it indicates one manner in which the metabolic block may have diverse and far reaching consequences.

In approaching therapy, it is more rational to lower the phenylalanine than to raise tyrosine. The latter appears adequate to correct pigment metabolism, but the former also removes the excesses in other metabolites such as phenylpyruvic acid and phenyllactic acid. It is not clear at present what the cause of the most significant abnormality, mental retardation, is. So it is wisest to return the patient, as far as possible, towards biochemical normality.

For a dietary approach to be effective, the infant must be born unharmed by the metabolic anomaly. If our current concepts of the disease and of placental function are accurate, this is likely to be the case. Transfer across the placenta is probably controlled by the relative levels of the amino acid in the fetal and maternal circulations. Phenylalanine will therefore be transferred to the fetus only in amounts sufficient to

TABLE II—PLASMA AMINO ACID ANALYSIS (mg. %)

	Patient	*Normal Subject*
Leucine	14.5	1.8
Isoleucine	2.2	1.2
Valine	13.5	3.2
Methionine	2.7	0.4
Cystine	0.0	0.5
Alanine	0.6	2.4
Serine	0.9	1.3
Threonine	0.3	1.2

keep pace with fetal metabolism, and will never pile up in excess. It would, in effect, result in an accurately controlled low phenylalanine diet. Problems would not arise until the pediatrician takes over for the placenta.

An interesting insight into genetics has also been obtained through the study of phenylketonuria. Orthodox genetic investigations had indicated that phenylketonuria is carried by a recessive gene, with an incidence of roughly one in one hundred leading to manifest disease in approximately one in forty thousand. Recently, by using phenylalanine load tests—similar in technique to the glucose tolerance test—a minor defect in handling phenylalanine has been demonstrated in the recessive[7]. This type of observation has modified our concept of the recessive from that of a completely normal individual who merely passes on the abnormal gene, to that of an individual who has the defect in so minor a form as to be clinically undetectable.

MAPLE SUGAR URINE DISEASE

Now, let us pass to the consideration of maple sugar urine disease. Clinically, maple sugar urine disease is also familial, causes mental retardation and a peculiar odor in the urine—this time the odor resembles that of maple sugar[8].

The first break-through in understanding the mechanism of the disease was the demonstration of a large leucine spot in the urine by Westall, who was then working at Bellevue Hospital. Investigation of

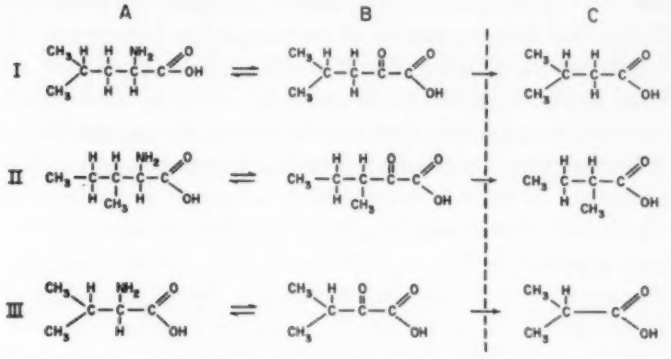

Fig. 2 — Metabolism of branched amino acids.

I. Leucine, II. Isoleucine and III. Valine

A. Amino acid ⟶ B. Keto-acid ⟶ C. Simple branched acid

Point of suspected block is between B & C.

the plasma amino acids revealed an elevation of the branched amino acids—leucine, isoleucine and valine—indicating the presence of a metabolic abnormality rather than a "renal leak". There were many other abnormalities in the amino acid pattern (Table II) but our attention focussed on this because the common configuration of these three amino acids suggested a common point of block.

Consideration of the normal metabolic pathway (Figure 2) indicated that oxidative-deamination or transamination was involved in the first step. Accordingly, when tissues became available from a patient at post-mortem, the presence of transaminase activity was investigated. Transaminase activity was found for the branched chain amino acids in all tissues studied. This indicated that the block must be further along the degradative pathway. Confirmation of this was soon provided by Menkes, working with urine on the same patient who demonstrated the presence of the respective keto-acids.

With the generous assistance of Dr. Lipsky of Yale University, we have analyzed the organic acids in the urine with gas phase chromatography. Several peaks were demonstrated, none of which corresponded to isobutyric or isovaleric acids (see Figure 2). This observation must be repeated. If confirmed, and I expect it will, it would localize the block to that of oxidative-decarboxylation of the keto-acids, and would

indicate that these 3 keto-acids share a common metabolic step. It is believed that decarboxylation of *a*keto-acids involves a cycle of reactions, interference with any one of which might result in such a block.

Many questions remain to be answered. Why do these patients get into trouble? It is unlikely that it is deficiency of materials beyond the block because they are relatively simple compounds and can probably be synthesized *de novo*. It is more likely an excess of the keto-acids or their derivatives that in some way interferes with metabolism. If so, will a dietary approach be effective? What about the genetic background of the disease? Load tests may be informative, though the relative rarity of the disease (or its recognition)* makes this approach difficult. Are there likely to be other keto-acidurias? Oxidative-deamination is the common first step in degradation of amino acids, and so any block in the next metabolic step should lead to a spill of the respective keto-acid. This may be a fruitful area for further research.

It is obvious that the approach, and even the framing of the questions, have been vastly simplified by the accumulation of experience gained from studying phenylketonuria and similar metabolic anomalies.

A preliminary report on the investigations of Maple Sugar Urine Disease will shortly be made by Dancis, J., Levitz, M., Miller, S. and Westall, R.

* It is of interest that Dr. James Dick of Long Island has diagnosed two cases in two unrelated families in his practice.

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DRUG-INDUCED HEMOLYTIC ANEMIAS AND CONGENITAL GALACTOSEMIA*

EXAMPLES OF GENETICALLY DETERMINED DEFECTS IN ERYTHROCYTE METABOLISM

PAUL A. MARKS and RUTH T. GROSS**

THIS discussion of "in-born errors" of erythrocyte metabolism is to be limited to a consideration of drug-sensitive hemolytic anemias and congenital galactosemia. Recent studies have established that both of these clinical syndromes are associated with specific erythrocyte enzymatic deficiencies.

DRUG-SENSITIVE HEMOLYTIC ANEMIAS

In 1948, Earle[1] observed the occurrence of hemolytic anemia in five to ten per cent of American Negro troops who had received pamaquine in the therapy of malaria. Similar experiences were reported following the use of primaquine[2]. These hemolytic episodes were observed very rarely among American Caucasian troops. Alving and his co-workers[3], studying the mechanism of primaquine-induced hemolytic anemia, observed that the hemolysis is self-limited. The lysis of red cells is a function of cell age, the older cells being the more susceptible[4]. Red cells of sensitive individuals are normal morphologically and have a normal life span unless challenged by a suitable agent[5]. These cells have no detectable abnormal antibodies and no disturbance either in osmotic or mechanical fragility[5].

Defects in Erythrocyte Metabolism. The erythrocytes of drug-sensitive subjects do have intrinsic metabolic defects. Figure 1 illustrates certain of the reactions of erythrocyte metabolism which are affected, either directly or indirectly, by the biochemical abnormalities in red cells of these subjects. Erythrocyte glucose utilization involves the phos-

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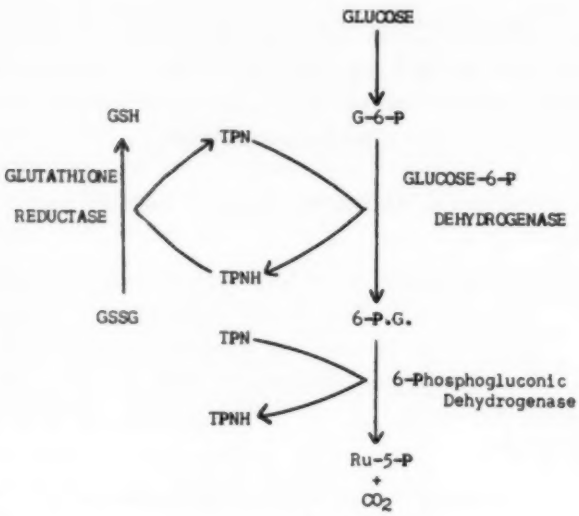

Fig. 1—Schematic representation of certain reactions of erythrocyte metabolism which are affected by the defect present in red cells of subjects with a genetically determined predisposition to drug-induced hemolytic anemias. Abbreviations employed: G-6-P, glucose-6-phosphate; 6-P.G., 6-phosphogluconic acid; Ru-5-P, ribulose-5-phosphate; TPN, triphosphopyridine nucleotide; TPNH, reduced triphosphopyridine nucleotide; GSSH, oxidized glutathione; GSH, reduced glutathione.

phorylation of glucose to glucose-6-phosphate which in turn may be metabolized either via the reactions of anaerobic glycolysis or of the pentose phosphate pathway[6, 7]. The first of the oxidative reactions of the pentose phosphate pathway results in the conversion of glucose-6-phosphate to 6-phosphogluconic acid. This reaction is catalyzed by the enzyme glucose-6-phosphate dehydrogenase, requires as a co-factor, triphosphopyridine nucleotide (TPN) and generates reduced TPN (TPNH). 6-Phosphogluconic acid is then oxidized to the five carbon sugar, ribulose-5-phosphate. In this reaction, another molecule of TPN is reduced to TPNH and CO_2 is generated by the oxidation of the first carbon of glucose. These reactions represent the only pathway in mature erythrocytes for the production of reduced TPN and for the oxidation of glucose to CO_2[8, 9]. Red cells possess the enzyme glutathione

reductase which catalyzes the conversion of glutathione (GSSG) to reduced glutathione (GSH). This reaction requires as a co-factor, TPNH, and results in the generation of TPN[10].

The concentration of reduced glutathione in erythrocytes of drug-sensitive subjects is generally lower than that of normal persons[11], though there is considerable overlapping of values[12, 13]. Beutler[14] has described a "reduced glutathione stability test" which permits the detection of primaquine-sensitive individuals by an *in vitro* assay. This test is based on the finding that following the *in vitro* incubation of red cells with acetylphenylhydrazine, erythrocytes from drug-sensitive, but not from normal, subjects show a marked decrease in the concentration of reduced glutathione. Carson and his co-workers[15] demonstrated that the erythrocytes of drug-sensitive persons are deficient in the enzyme glucose-6-phosphate dehydrogenase. This finding of deficiency in red cell dehydrogenase was confirmed[16, 17] and found to be associated with a reduced capacity of these erythrocytes to oxidize glucose to CO_2, as well as a lowered rate of oxygen consumption when incubated in the presence of methylene blue[9]. Schrier and his co-workers reported that these erythrocytes have increased concentrations of the enzymes, glutathione reductase[18] and aldolase[19] and of TPN[20].

The most marked biochemical deviation from normal in the red cells from sensitive subjects appears to be the decrease in glucose-6-phosphate dehydrogenase. The other alterations in the metabolism of these erythrocytes could be secondary to the dehydrogenase deficiency. Thus, the reduction in glucose-6-phosphate dehydrogenase might be associated with a decreased rate of TPNH formation. A deficiency in TPNH would limit the rate of GSH generation and could account for the apparent instability of reduced glutathione. It has been suggested that the increase in glutathione reductase activity may represent a compensatory phenomenon, providing for increased concentrations of TPN and for more efficient GSH formation[12].

Substances Which May Induce Hemolytic Anemias. The elucidation of these biochemical abnormalities in erythrocytes of drug-sensitive subjects has not provided an explanation of the mechanism of the increased hemolysis following the ingestion of various agents. The list of such agents associated with hemolytic anemia is ever increasing and now includes nitrofurantoin[21], sulfanilamide[22, 23], acetanilid[22], various vitamin K substitutes[24], phenacetin[22], naphthaline[16, 25], fava bean[16, 17, 27, 28]

and isolated cases have been observed associated with the ingestion of para-amino salicylic acid, isioniazid and salicylic acid[29, 30].

Nitrofurantoin[21], certain vitamin K analogues and the naphthaline metabolites, γ and β naphthol[24], have been demonstrated *in vitro* to lead to a decrease in the reduced glutathione concentration but not hemolysis of erythrocytes from drug-sensitive individuals. However, sulfonamides and various preparations of the fava bean have not been shown to have similar *in vitro* effects[29]. Alving and his co-workers[12] have reported that administration of 30 mg. primaquine base (twice the daily therapeutic dose for vivax malaria) causes hemolysis in essentially all subjects with drug-sensitive erythrocytes. It should be emphasized, however, that subjects with deficient erythrocyte glucose-6-phosphate dehydrogenase may ingest certain of these agents without any apparent ill effects[29, 30]. In addition, patients who are unaffected by fava bean ingestion developed hemolytic anemia associated with sulfonamide administration[23]. The occurrence of increased hemolysis may in part be related to the dose of the potentially offending agent. In addition, different mechanisms may be involved in the hemolytic anemias induced by the different agents. It may be speculated that erythrocytes deficient in TPNH generation may be particularly susceptible to hemolysis because of reduced capacity to detoxify drug products or because of deficient synthetic processes, such as membrane lipid synthesis[6, 7].

Effect of Age on Erythrocyte Enzyme Activity. It is possible that some insight has been gained into a curious clinical aspect of the drug-induced hemolytic anemias, namely, their tendency to be self-limited and a function of cell age. It has been demonstrated in observations on normal subjects, that glucose-6-phosphate dehydrogenase activity is high in young erythrocytes and decreases markedly with the *in vivo* aging of these cells[31]. Studies in subjects with erythrocyte glucose-6-phosphate dehydrogenase deficiency have revealed that the level of this enzyme is higher in young, compared with old red cells[32]. The enzyme has been assayed in red cells of control subjects and persons whose erythrocytes had intermediate (2.5 to 4 standard deviations below the mean value for the control group) and low (less than 4 standard deviations below the mean for the control group) glucose-6-phosphate dehydrogenase activities. The whole erythrocyte population was separated into fractions of relatively young and old mean cell age by a method of serial osmotic hemolysis[33]. In subjects whose whole

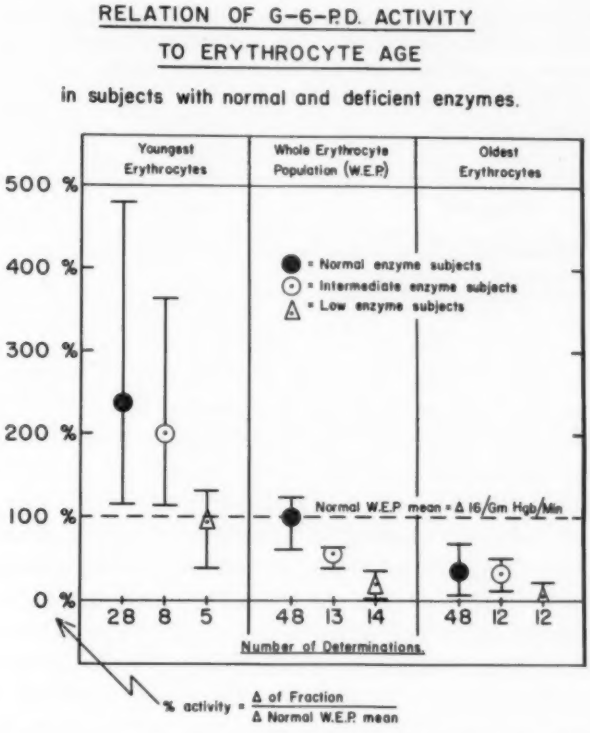

Fig. 2—The effect of age on the glucose-6-phosphate dehydrogenase activity of erythrocytes from subjects with normal, intermediate and low levels of dehydrogenase in the whole erythrocyte population. The mean level of enzyme for normal whole erythrocyte population is taken as equal to 100 per cent. The mean value plus and minus one standard deviation are represented for the whole erythrocyte population and youngest and oldest cell fractions for each of the three groups of subjects studied. The per cent activity is expressed as a per cent of the mean value for the normal whole erythrocyte population (Δ of fraction/ Δ normal W.E.P. mean). The methods employed to obtain these data are indicated in the text.

erythrocyte population was deficient in glucose-6-phosphate dehydrogenase, the level of this enzyme in young red cells, though not equal to normal levels, was distinctly higher than in old cells (Figure 2). No evidence was obtained for an inhibitor of glucose-6-phosphate dehydrogenase in hemolysates of erythrocytes deficient in this enzyme upon

TABLE I

INCIDENCE OF ERYTHROCYTE GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY IN A RANDOM POPULATION AND AMONG RELATIVES OF PROPOSITI

Random Population	*Subjects tested*	*% affected*
Total	666	7.3
Negroes	361	13.0
Caucasian	305	0.7
Family Studies		
Relatives of 31 propositi	154	40.0

incubation with hemolysates prepared from cells of normal subjects or with purified glucose-6-phosphate dehydrogenase[17, 32]. Thus, drug-induced hemolysis may cease when the older cells, deficient in dehydrogenase, have been destroyed. The remaining red cell population would be composed of young cells with higher levels of this enzyme and presumably relatively resistant to hemolysis. This hypothesis remains to be proved. It has been observed that following a drug-induced hemolytic episode, there is a rise in the level of erythrocyte glucose-6-phosphate dehydrogenase coincident with a reticulocytosis[30, 34, 35]. Such elevations in enzyme level have persisted for 40-80 days following the return of the reticulocyte count to within normal levels. Kimbro and co-workers[21] reported that readministration of nitrofurantoin at 35 and 75 days after the first course induced further hemolysis. However, Flanagan and associates[34] observed that in a single subject, given 30 milligrams of primaquine continuously for one year, no further detecable hemolysis occurred despite return of glucose-6-phosphate dehydrogenase to the pre-therapy low levels.

Genetic Studies. It is apparent that the identification of glucose-6-phosphate dehydrogenase deficiency and lowered concentrations of reduced glutathione as important defects in erythrocytes of drug-sensitive subjects has not as yet provided an explanation for the mechanism of their increased susceptibility to hemolysis. Nevertheless, they have provided important tools for studying the incidence and hereditary aspects of this "error" in erythrocyte metabolism.

In addition to the relatively high incidence of this defect among American Negroes[12-14, 36], it has been reported in Sephardic Jews[29],

TABLE II

SEX DISTRIBUTION OF SUBJECTS WITH GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENT RED CELLS[1]

	Middle[2]	*Low*[3]
Males	8	62
Females	48	3

Notes

1. This table includes subjects found to have erythrocytes deficient in glucose-6-phosphate dehydrogenase in the random population study and among relatives of propositi.
2. Middle refers to erythrocyte glucose-6-phosphate dehydrogenase activity between 2.5 and 4 standard deviations below the mean for the control group.
3. Low refers to erythrocyte glucose-6-phosphate dehydrogenase activity more than 4 standard deviations below the mean for the control group.

Italians[37, 38] primarily of Sicilian and Sardinian descent and subjects of Greek descent[13, 17]. In a random population of 666 subjects studied in the San Francisco and New York City areas, the observed incidence of erythrocyte glucose-6-phosphate dehydrogenase deficiency was 0.7 per cent among the Caucasians and 13.0 per cent among the Negroes (Table I). These findings are in general agreement with those of several other workers employing either the enzyme assay or the "glutathione stability test"[12, 14, 36, 39]. The bimodal distribution of this defect in a random population study suggests that the trait is genetically determined. An hereditary determination of this trait is strongly indicated by the finding of an incidence of 40 per cent among 154 relatives of 31 propositi (Table I). A similar incidence of affected relatives of drug-sensitive subjects was reported by Childs and co-workers[36] in the Baltimore area and by Szeinberg and Sheba[29] among Oriental Jews. Observations by several investigators are compatible with the trait being inherited as a sex-linked gene of intermediate dominance[13, 29, 36]. In families where the mother presented the abnormality, it was passed on to both the male and the female child, whereas affected fathers transmitted the defect only to daughters. Without exception, in 26 families studied to date, we have observed no incidence of a male with a low red cell dehydrogenase being born to a female with a normal level. Among parents of affected subjects there is a striking preponderance of mothers, 25 out of 29. These findings suggest that the trait is transmitted by a gene linked to the female sex chromosome.

TABLE III

DISTRIBUTION OF ERYTHROCYTE GLUCOSE-6-PHOSPHATE DEHYDROGENASE ACTIVITY AMONG A RANDOM POPULATION OF NEGROES

	Normal[1]	*Middle*[2]	*Low*[2]
Male[3]	177	0	17
Female	184	28	2

Notes

1. Erythrocyte glucose-6-phosphate dehydrogenase activity among normals was found to be 15.9 ± 2.4 change in optical density units per gram of hemoglobin[26].
2. See notes 1 and 2, Table II.
3. *Calculation of gene frequency*[39]:
   Assuming the trait is sex-linked, the genotype frequency (q) in the male population will be equal to the gene frequency. The frequency of the trait among female heterozygotes (2 pq) can be calculated from the observed incidence of the male hemizygote (q = .0971) in the male population (p = 1-q = .9029). This yields a calculated value for female heterozygotes (2 pq) in the above population equal to 17.5 per cent. The observed value was 16.3 per cent. The calculated and observed values are not significantly different ($p = >> .05$).

Subjects with low erythrocyte glucose-6-phosphate dehydrogenase may be divided into two groups on the basis of the severity of the enzymatic deficiency. Subjects with intermediate levels of enzyme (2.5 to 4 standard deviations below the mean for the control group) are predominantly affected females, whereas persons with low levels of enzyme (more than 4 standard deviations below the mean for the control group) are almost entirely males (Table II). It has been suggested[36] that the low and intermediate enzyme levels represent the homozygous and heterozygous states, respectively. However, we have observed in one family a mother with a very low level of glucose-6-phosphate dehydrogenase who gave birth to four normal sons. There are alternative explanations for the sex distribution of affected subjects. These include 1) that the phenotypic expression is modified in the female or 2) that we are dealing with a characteristic determined by more than a single genetic defect, i.e., a genetically heterogenous trait.

The frequency of the trait among heterozygous females may be predicted from calculations based on the incidence of male hemizygotes in a population[39]. In this calculation, the assumption is made that the trait is sex-linked. In a sex-linked trait, the genotype frequency among males will equal the gene frequency. Such a calculation (Table III, Note 3) indicates that there is excellent agreement between the pre-

TABLE IV

GLUCOSE-6-PHOSPHATE DEHYDROGENASE ACTIVITY IN LEUKOCYTES (WBC) AND RED CELLS (RBC) OF CONTROL SUBJECTS AND PERSONS WITH A DEFICIENCY OF THIS ENZYME IN THEIR CIRCULATING ERYTHROCYTES

Subjects	*Number*	*WBC**	*RBC*
Control	31	37.3 ± 5.8	0.49 ± 0.11
Deficient Red Cell G-6-P.D.**	14	36.8 ± 1.8	0.07 ± 0.01

Notes

*The unit of enzyme activity is expressed as the change in optical density per minute per 10^6 cells (Δ OD/min/10^6 cells). The mean values plus or minus one standard deviation are given in this table.

**G-6-P.D. is glucose-6-phosphate dehydrogenase. All subjects included in this group had erythrocyte glucose-6-phosphate dehydrogenase levels below three standard deviations of the mean for the control group.

dicted and actually observed incidence of low enzyme levels among females. This finding suggests that the enzyme assay provides a relatively accurate tool for the detection of the heterozygous state and, hence, the potential carrier of this defect.

The enzyme assay also affords a ready means of screening subjects for an increased susceptibility to drug-induced hemolytic anemias. Pertinent to the use of such an *in vitro* screening test are the observations of Alving and his co-workers[12] that the "reduced glutathione stability test" does not accurately reflect susceptibility to increased hemolysis following primaquine administration. Although we have found a high degree of correlation between the "glutathione stability test" and the level of glucose-6-phosphate dehydrogenase in erythrocytes, certain subjects were observed whose enzyme activity was decreased, yet whose *in vitro* glutathione stability test was within normal levels[13]. Further evaluation of erythrocyte glucose-6-phosphate dehydrogenase activity as an index of susceptibility to increased hemolysis must await studies correlating *in vitro* assays of red cell glucose-6-phosphate dehydrogenase with drug-induced hemolytic episodes.

Enzyme Activity in Tissues of Subjects with Glucose-6-Phosphate Dehydrogenase Deficient Erythrocytes. Glucose-6-phosphate dehydrogenase has been assayed in leukocytes of subjects whose erythrocytes are deficient in this enzyme[40]. The level of dehydrogenase in the leukocytes from these subjects did not differ from that in the white

cells of normal subjects (Table IV). In addition, a subject whose erythrocyte glucose-6-phosphate dehydrogenase was markedly deficient was not found to have a similar decrease in his liver enzyme activity. These findings are relevant to the question of the relationship between the genetic defect and the phenotypic expression which is measured as the level of glucose-6-phosphate dehydrogenase. If a suppression in the synthesis of this enzyme was the direct effect of the abnormal gene, all tissues of the affected subject would be expected to exhibit a similar enzyme deficiency. Accordingly, these data suggest that erythrocyte glucose-6-phosphate dehydrogenase is not a primary, but rather is a more remote consequence of the presence of a genetic defect.

Summary of Current Concepts of "Drug-Induced" Hemolytic Anemias:

1. Drug-induced hemolytic anemias are associated with certain intrinsic defects of erythrocyte metabolism, chief among which may be a deficiency in glucose-6-phosphate dehydrogenase.
2. Detection of susceptible individuals, as well as genetic carriers, is possible by assay *in vitro* of glucose-6-phosphate dehydrogenase levels in red cells and perhaps, with a lesser degree of accuracy, erythrocyte reduced glutathione stability.
3. The defect in erythrocyte metabolism seems to be determined genetically and may be transmitted as a sex-linked trait of intermediate dominance. The nature of the primary effect of the abnormal gene is unknown.
4. The mechanism of the hemolytic anemia remains to be elucidated.

Congenital Galactosemia

The genetically determined defect in the metabolism of the erythrocytes of subjects with galactosemia reflects an enzymatic deficiency, the consequences of which are not restricted to the erythrocyte, as may be the case for the defect in the drug-sensitive hemolytic anemias. Galactosemia is a congenital and hereditary disorder, characterized by difficulty in the metabolism of ingested galactose or milk. Ingestion of galactose by these patients is associated with the development of impaired function of the liver and spleen, of cataract formation, and of mental retardation[41]. The laboratory usually reveals marked galactosuria and an abnormal galactose tolerance test. As the disease pro-

gresses, hepatic insufficiency and evidence of renal functional impairment are frequent complications. The elimination of galactose from the diet may lead to improvement and even disappearance of symptoms.

Galactose Metabolism. The metabolism of galactose involves the following series of reactions[42, *]:

1) galactose + ATP $\xrightarrow{\text{galactokinase}}$ galactose-1-phosphate + ADP.

2) galactose-1-phosphate + UDPG $\xrightarrow[\text{urydil transferase}]{\text{galactose-1-phosphate}}$ UDPGal + glucose-1-phosphate.

3) UDPGal $\xrightarrow{\text{epimerase}}$ UDPG.

Galactose is phosphorylated to form galactose-1-phosphate in a reaction catalyzed by an enzyme called galactokinase and requiring, as a source of high energy phosphate, ATP. In reaction 2, galactose-1-phosphate is converted to glucose-1-phosphate. This reaction involves, as a co-factor, UDPG. The resulting glucose-1-phosphate is not derived directly from galactose-1-phosphate. The glucose-1-phosphate is formed from UDPG and galactose-1-phosphate becomes attached to the nucleotide resulting in UDPGal. The enzyme catalyzing this reaction is referred to as galactose-1-phosphate urydil transferase. In reaction 3, UDPGal may be directly converted to UDPG. The only structural difference between the two hexoses, galactose and glucose, is in the orientation of the hydrogen and hydroxyl groups about the fourth carbon atom. The mechanism of reaction 3 is believed to involve the removal of two hydrogen atoms from the fourth carbon atom of the hexose, presumably forming a ketone derivative. These two hydrogen atoms may then be returned to the hexose with the formation of the opposite compound or epimere, UDPG, when the starting compound is UDPGal. The enzyme catalyzing this reaction is called epimerase. The net result of this series of reactions is to permit the entrance of galactose into the general carbohydrate metabolism by converting it to glucose-1-phosphate and providing for the regeneration of the necessary co-factor, UDPG.

Enzyme Defect in Galactosemia. In 1956 Schwarz and his co-workers[43] reported that feeding of galactose or milk to infants with

* Abbreviations employed: ATP, adenosine triphosphate; UDPG, uridine diphosphate glucose; UDPGal, uridine diphosphate galactose; UTP, uridine triphosphate.

TABLE V

ENZYME ACTIVITY IN ERYTHROCYTES OF SUBJECTS WITH CONGENITAL GALACTOSEMIA[46]

Enzyme	*μ Moles/ml/hr.* *Normal*	*Galactosemia (12 cases)*
Galactokinase	0.12	0.10
Galactose-1-Phosphate Urydil Transferase	0.75	0.00
Epimerase	0.32	0.40

congenital galactosemia was associated with the accumulation of galactose-1-phosphate in their red cells. Such an accumulation did not occur in erythrocytes of normal subjects. These observations were confirmed and extended by Kalckar, Anderson and Isselbacher[44] who demonstrated that erythrocytes from galactosemic subjects incubated *in vitro* with galactose accumulated galactose-1-phosphate while red cells from normal subjects did not. These investigators suggested that the defect in galactosemia might reside in the enzyme catalyzing reaction 2, urydil transferase. A deficiency of this enzyme could account for the accumulation of galactose-1-phosphate following administration of galactose.

Employing specific assays for the three enzymes catalyzing reactions 1 through 3, it was demonstrated that only the enzyme, galactose-1-phosphate urydil transferase, was deficient in erythrocytes from galactosemic subjects compared to those of normal controls (Table V)[45].

Identification of this specific enzymatic defect in erythrocytes of galactosemic subjects provides a specific and relatively easy diagnostic test for this condition. The enzymatic assay avoids any hazard involved in the galactose tolerance test. In addition, it can be done at birth, on a small amount of blood.

Genetic Studies. Unlike the findings in subjects with erythrocyte glucose-6-phosphate dehydrogenase deficiency, two persons with congenital galactosemia have been demonstrated to lack the transferase in liver tissue in addition to red cells[47]. Thus, the present data are compatible with galactosemia being due to a hereditary defect in which the primary action of the gene may be a suppression of synthesis of galactose-1-phosphate urydil transferase.

The frequent occurrence of galactosemia among sibs and offspring of consanguineous matings together with its equal distribution in both sexes has suggested that galactosemia is probably transmitted as a single autosomal recessive gene. The disease is presumably clinically apparent only in the homozygous state. It would be expected that if galactosemia were inherited as a recessive gene, both parents should be heterozygous and although clinically asymptomatic might give evidence of a deficiency in galactose-1-phosphate urydil transferase. On the basis of both galactose tolerance tests[48] and assays for the enzyme[49, 50], it would appear that some parents of galactosemic children have a detectable impairment in galactose metabolism and others do not. This failure to detect impairment among some subjects who are presumably heterozygous for the galactosemic defect may merely reflect a degree of variation in the level of enzyme with a distribution curve among heterozygotes that overlaps, on the high side, that of normal subjects. It has also been suggested[49] that these findings might be due to the presence of a suppressor gene or the fact that more than one genetic defect may cause the clinical picture of congenital galactosemia.

Mechanism of Symptomatology and Improvement with Age. The demonstration of a deficiency in galactose-1-phosphate urydil transferase in patients with congenital galactosemia neither explains the mechanism of the varied symptomatology of this disease, nor the curious clinical observation that patients with congenital galactosemia generally improve with age. This improvement is associated with an increased ability to metabolize galactose, as reflected in an improvement in their galactose tolerance test[51].

Studies in animals suggest that the accumulation of galactose-1-phosphate may be toxic and lead to abnormal neurological manifestations[52]. In addition, there is evidence to indicate that the accumulation of galactose-1-phosphate may interfere with glucose utilization and lead to metabolic derangements which may have relevance to the cause of the clinical symptoms. Thus, galactose-1-phosphate has been found to inhibit *in vitro*, the enzymes, phosphoglucomutase[54, 55] and glucose-6-phosphatase[46]. It also has been found that mutants of Escherichia coli which accumulate galactose-1-phosphate due to lack of galactose-1-phosphate urydil transferase show retardation of growth in the presence of galactose plus glucose compared to their growth in the presence of glucose without galactose[53].

The improved galactose metabolism in patients associated with increased age, may be explained on the basis of the recent demonstration by Isselbacher[56] that there is an additional pathway of galactose metabolism which permits the incorporation of galactose into uridine diphosphate galactose and, in turn, to form glucose derivatives. This pathway involves the direct conversion of galactose-1-phosphate to uridine diphosphate galactose by reaction with the nucleotide, uridine triphosphate:

$$\text{UTP} + \text{galactose-1-phosphate} \xrightarrow{\text{UDPGal pyrophosphorylase}} \text{UDPGal} + \text{pyrophosphate.}$$

It has also been found[57] that UDPGal pyrophosphorylase is very low in fetal and neonatal tissues compared with adult tissues. Further, in normal tissue the activity of this enzyme is only about one-sixth of that of the galactose-1-phosphate urydil transferase. Thus, it would appear that in normal subjects the galactose-1-phosphate urydil transferase pathway is the one of primary importance. However, in galactosemic patients who appear to lack this enzyme, the observation that the alternate pathway of galactose metabolism may increase in activity associated with aging could explain the improvement in symptoms in galactosemic patients as they grow older.

Summary of Current Concepts of Galactosemia.

1. Galactosemia appears to be a congenital disease associated with a specific enzymatic defect, galactose-1-phosphate urydil transferase deficiency.
2. The identification of this specific enzyme defect in erythrocytes provides a simple diagnostic tool for the detection of this syndrome.
3. The symptomatology associated with galactosemia may be related to the accumulation of galactose-1-phosphate in the tissues.
4. The improvement in galactose tolerance test and symptomatology with age may be referable to the development and utilization of an alternate pathway of galactose metabolism.

Drug-induced hemolytic anemia and congenital galactosemia are only two examples of genetically determined defects in metabolism which are reflected in biochemical alterations in the readily accessible erythrocyte. Undoubtedly, this is a fertile field for further investigations in the area of "in-born" errors of metabolism.

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GILBERT'S DISEASE*

IRWIN M. ARIAS**

IN 1900 Gilbert and his associates described a series of patients with chronic familial nonhemolytic acholuric jaundice of varied clinical intensity[1]. Many of Gilbert's patients probably had what today we recognize as compensated hemolytic states; however, the entity of nonhemolytic acholuric jaundice which he described has been shown to exist in several forms and may be classified in the following manner.

1. In 1952 Crigler and Najjar[2] described seven infants in three related family groups who developed nonhemolytic acholuric jaundice shortly after birth with indirect-reacting serum bilirubin concentrations of 13 - 45 mgm. per cent. Kernicterus developed in all but one child during the first few months of life and five of the children died before reaching the age of one year. Other sporadic cases of a similar nature have been observed.

2. The entity variously described as Constitutional Hepatic Dysfunction or Physiologic Hyperbilirubinemia is usually discovered in early adolescence. The serum indirect-reacting bilirubin concentration is usually less than 5 mgm. per cent and there are no serious sequelae of the disease. In addition there are occasional cases of a similar benign nature except that the serum bilirubin concentration may be as high as 15 mgm. per cent.

3. Nonhemolytic acholuric jaundice is also manifested by the majority of full term infants between the second and seventh days of life. When this situation prevails in the premature infant, the levels of indirect-reacting bilirubin may be considerably higher and kernicterus may result.

4. The last type of nonhemolytic acholuric jaundice is that occurring in a mutant strain of rats first described by a Canadian geneticist, Gunn[3], in 1938. The homozygous rats become jaundiced shortly after

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birth and often develop kernicterus. These homozygous animals appear to be prototypes of the syndrome described by Crigler and Najjar. Malloy and Lowenstein[4] observed an inability of these animals to excrete infused bilirubin at a normal rate, an observation akin to that noted in human children and adults with nonhemolytic acholuric jaundice of the various types.

A study of the mechanism of jaundice in these various states involves an understanding of the processes by which bilirubin is transported from serum protein through the liver cells and into the bile. Prior to 1953 it was often claimed that the liver removed bilirubin from "bilirubinglobin" (the presumed "indirect-reacting" bilirubin) and excreted it in the bile as the sodium salt, which was believed to be responsible for the "direct-reaction" with diazotized sulphanilic acid. In 1953 Cole and Lathe[5] isolated preparations of bilirubin which, free of protein, yielded either the "direct" or "indirect" van den Bergh reaction. More recently Billing and Lathe[6] in England, Talafant[7] in Czechoslovakia, and Schmid[8] in this country independently demonstrated that most of the bilirubin which gives the "direct reaction" in freshly obtained bile or in serum or urine from patients with obstructive jaundice, is conjugated with glucuronic acid. Isselbacher and McCarthy[9] have shown that bilirubin sulfate constitutes approximately 15 per cent of the total bilirubin conjugates of normal human bile and that an additional 9 per cent of other carboxyl-linked, alkali-labile derivatives also occur. The glucuronide conjugation occurs on the carboxyl group of the propionic acid substituent of the bilirubin molecule forming an acyl or ester linkage[7, 10]. Unconjugated bilirubin is responsible for the "indirect" van den Bergh reaction.

A substance present in boiled liver extract, later shown to be uridine diphosphate glucuronic acid (UDPGA), was found by Dutton and Storey[11] to enhance the formation of glucuronides of various receptors by rat liver homogenates. As is indicated in the following reactions, UDPGA is formed by oxidation of uridine diphosphate glucose (UDPG) by a specific enzyme, UDPG dehydrogenase, which was found in the soluble fraction of rat liver homogenate by Strominger and his associates[12]. The glucuronic acid used in conjugation by this pathway is ultimately derived from glucose as indicated in the first reaction. The microsomal fraction of rat liver homogenate contains enzymatic activity, designated as glucuronyl transferase activity, which

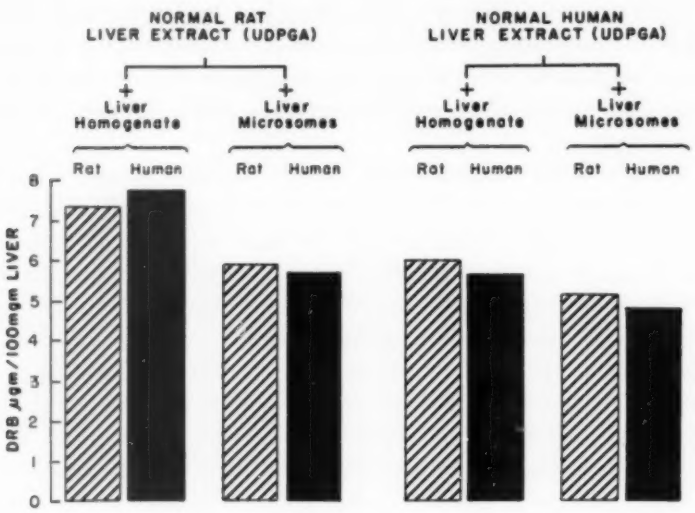

Fig. 1

"Direct reacting" Bilirubin Production by Normal Rat and Human Liver Preparations.

catalyzes the transfer of glucuronic acid from UDPGA to various receptors indicated by "R" in reaction 3[11, 12].

1. Glucose-1-Phosphate + UTP $\rightleftharpoons$ UDPG + PP
   (P Uridyl Transferase)
2. UDPG + DPN^+ $\rightleftharpoons$ UDPGA + DPNH
   (UDPG Dehydrogenase)
3. UDPGA + "R" $\rightleftharpoons$ "R" GA + UDP
   (Glucuronyl Transferase)

The *in vitro* biosynthesis of bilirubin glucuronide was based upon reaction 3, namely, the transfer of glucuronic acid from UDPGA to bilirubin by the enzyme(s) glucuronyl transferase(s) which is present in the microsomal fraction of liver homogenates of man and several animal species. The *in vitro* system consists of 1) bilirubin, 2) UDPGA as provided by a boiled liver extract and in later experiments by purified UDPGA, and 3) homogenates of rat or human liver or the microsomal fractions prepared from these homogenates.

TABLE I—DATA CONCERNING PATIENTS WITH NONHEMOLYTIC ACHOLURIC JAUNDICE

	B.H. $\binom{O}{+}$	M.S. $\binom{O}{+}$
Age	21	43
Family History of Icterus	Yes	Not Known
Consanguinity of Parents	Yes	Not Known
Mental Status	Normal	Idiot
Neurological Status	Normal	Signs of Extra-Pyramidal Disease
Serum Bilirubin Conjugated	0.00 mgm. %	0.00 mgm. %
Non Conjugated	8.8 mgm. %	18.8 mgm. %
Liver Morphology	Normal	Normal
Fecal Urobilinogen Excretion	———	Decreased

Both Patients had Normal Hemoglobin Concentration, R.B.C. Osmotic Fragility, Reticulocytes, Bromsulphalein Retention, Cephalin Flocculation, Thymol Turbidity, Urine Urobilinogen and Extrahepatic Biliary System. There was no Bilirubinuria.

Figure 1 illustrates the production of "direct-reacting" bilirubin glucuronide when liver extracts and homogenates or microsomal fractions of rat and normal human liver were used interchangeably as sources of UDPGA and glucuronyl transferase activity. This system has been applied to the study of some of the previously mentioned disease states[13].

Liver tissue was obtained at operation from two patients with nonhemolytic acholuric jaundice (Table I). The first was 21 years of age; the second was 43. Both had been icteric since birth. The second patient had evidence of disease of the basal ganglia and was mentally defective. Neither had anemia nor other abnormal liver function tests. There was normal urine urobilinogen excretion, no bilirubinuria and, in the second patient, fecal urobilinogen was approximately 40 per cent of normal. The serum unconjugated bilirubin concentrations were 8.8 and 18.8 mgm. per cent respectively. No conjugated bilirubin was detected in either serum.

Figure 2 illustrates the production of "direct-reacting" bilirubin when either liver of the rat or of the patient served as a source of UDPGA and *rat* liver homogenate or microsomes served as a source of glucuronyl transferase activity. When either the *human* liver homo-

"Direct Reacting" Bilirubin Production by Liver Preparations of Patients* with Nonhemolytic Acholuric Jaundice

Fig. 2

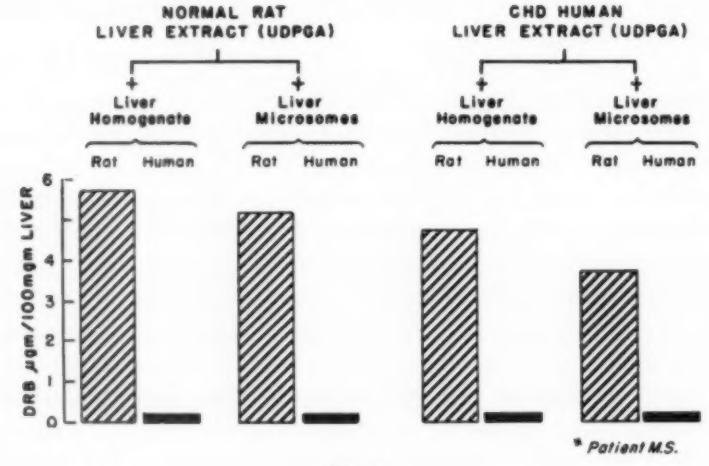

Fig. 3

TABLE II—GLUCURONIDE SYNTHESIS BY MALE GUNN RAT LIVER MICROSOMES

Genetic Type	*No. of Rats*	*0-Aminophenol Gluc. Formed (*)*	*No. of Rats*	*Bilirubin Glucuronide Formed (*)*
Homozygous	4	0.000 - 0.012	3	0.000
Heterozygous	6	0.025 - 0.029	5	0.011 - 0.039
Homozygous Normal	8	0.067 - 0.074	6	0.056 - 0.089

*μmoles glucuronide formed per gm. wet weight of liver

genate or microsomes served as a source of transferase activity, there was virtually no production of conjugated bilirubin.

Figure 3 illustrates similar findings in the second patient, namely, the absence of detectable glucuronyl transferase activity in the liver homogenate or microsomal preparation from the patient with nonhemolytic acholuric jaundice.

An inhibitory effect of the human homogenates could not be demonstrated. The data suggest that the defect in these patients may be a deficiency of glucuronyl transferase activity. Presumably this defect results in an inability to form the water soluble bilirubin glucuronide which is readily excreted in the bile and urine whereas unconjugated bilirubin is not. It is likely that this defect accounts, at least in part, for the resulting hyperbilirubinemia in these patients.

Observe that the excretion of bile pigment is markedly decreased, presumably due to the enzymatic defect in glucuronide formation. The serum bilirubin concentrations did not vary by more than 20 per cent during the patients' lifetime. The tissue bilirubin did not apparently increase, as evaluated by gross clinical inspection. Red blood cell survival and hence bilirubin formation were normal. Where does bilirubin go in these patients? How is it metabolized or excreted? These important questions are at present unanswered.

When studied *in vivo*, patients with unconjugated bilirubinemia over 15 mgm. per cent have been shown to have an impaired capacity to form glucuronides of salicylate, menthol and other substances normally conjugated with glucuronic acid[14, 15].

A deficiency in glucuronyl transferase activity also occurs in the livers of the jaundiced Gunn rats in which the excretion of bilirubin

TABLE III—PATIENTS WITH NONHEMOLYTIC ACHOLURIC JAUNDICE

Patient (& Age)	*Serum Unconjugated Bilirubin (mgm. %)*	*Family History of Jaundice*	*Fecal Urobilinogen Excretion*
M.S. (43)	18.8	———	Decreased
P.H. (14)	13.5	Yes	———
H.J. (33)	10.5	Yes	Decreased
R. de L. (9)	9.0	Yes	———
B.H. (21)	8.8	Yes	———
N.H. (17)	6.2	Yes	———
V.H. (20)	5.7	Yes	———
J.A. (7)	5.5	No	———
R.R. (29)	4.1	———	Normal
9 Patients (5-19)	1.4-3.9	Yes (3 Patients)	Normal (2)

in the bile is also decreased (Table II)[14, 16, 17]. The heterozygous animals are not icteric and assay of liver glucuronyl transferase reveals activity between 30 and 50 per cent of that of the homozygous normal animals of the same strain. UDPGA formation is unimpaired in all animals of this strain. Similarly in livers of human fetuses and newborn humans and guinea pigs, a deficiency of liver glucuronyl transferase activity has been demonstrated which persists until about the tenth day of life[17, 18]. Brown and Zuelzer[18] have shown that the UDPG dehydrogenase activity is also decreased in livers of newborn guinea pigs with the result that there is diminished UDPGA formation during the first ten days of life. These observations suggest that the physiologic jaundice of newborns and prematures may be related to delayed development of the glucuronide conjugating system.

Whether all forms of nonhemolytic acholuric jaundice, as seen beyond the neonatal periods, represent the same biochemical defect in glucuronide formation is currently under investigation. The use of the eponym "Gilbert's Disease" has resulted in confusion in this regard. Some investigators have arbitrarily limited its use to those patients with bilirubinemia of up to about 4 mgm. per cent. Others have included patients with all degrees of bilirubinemia within this category pending subclassification based upon further study.

Schmid[19] has recently shown that patients with bilirubinemia up to 3 mgm. per cent excrete normal amounts of N-acetyl-para-aminophenol glucuronide after administration of the test material. Because of these

observations an alteration in hepatic cellular uptake of bilirubin has been proposed as the basis for the jaundice in these patients.

I should like to present observations which provide further evidence for deficient glucuronyl transferase activity in the livers of patients with nonhemolytic acholuric jaundice. These observations suggest that there is a spectrum of the disease and that the several entities in adults may represent varied degrees of expressivity of the same functional defect.

Our experience is based upon the following group of patients who have been referred to us by many hospitals and physicians. Table III presents observations on 18 patients with nonhemolytic acholuric jaundice. Hemolysis was excluded by either *in vivo* red blood cell survival study (seven patients), normal osmotic fragility curve (17 patients) and absence of anemia or reticulocytosis (18 patients). In no instance was there detectable azo-bilirubin glucuronide in the serum. All of the serum bilirubin was accounted for by unconjugated bilirubin. Routine liver function studies including bromsulfalein retention tests were normal. The extrahepatic biliary system was normal as observed either at surgery or by intravenous cholangiography. The liver was histologically normal on examination of liver biopsy by routine histologic methods. The family history was either not available or was unreliable in three patients. Six patients denied any family history of acholuric jaundice. The remaining ten patients gave a history of such jaundice in their families and in six instances it was possible to document the observation. The available data are not complete enough to warrant a discussion regarding the precise genetic transmission of the disease. The most striking familial pattern is evidenced by a family in which a mother and two daughters have nonhemolytic acholuric icterus of varied severity with a history of a maternal aunt having had "yellow eyes" for most of her life. It has not been possible to detect nonicteric carriers on the basis of bilirubin tolerance tests which have been performed in four siblings of these patients. *In vivo* studies of glucuronide conjugation in these particular patients have not been completed.

Glucuronyl transferase activity has been assayed in liver tissue obtained by standard aspiration biopsy from ten of these patients. In order for this to be done it was necessary to develop a method which would enable accurate and reproducible assays to be performed on small amounts of liver. A receptor other than bilirubin was required

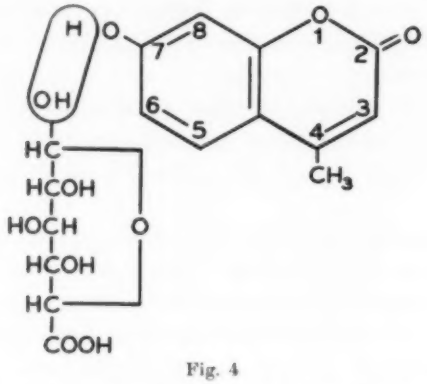

Fig. 4

4-Methyl Umbelliferone (4-methyl-7-hydroxy coumarin) Glucuronide

because the methods for studying bilirubin glucuronide formation are applicable only to larger amounts of liver. At the suggestion of Doctor Gordon Tompkins of the National Institutes of Health, 4-methyl umbelliferone was used as a glucuronide receptor and an assay procedure was developed which is applicable to liver tissue obtained by conventional aspiration biopsy[20].

Chemically, 4-methyl umbelliferone is 4-methyl-7-hydroxy coumarin and it forms an ethereal glucuronide as illustrated in Figure 4. The unconjugated substance is highly fluorescent. Fluorescence disappears when the glucuronide is formed. The disappearance of fluorescence has been quantitated to estimate the formation of umbelliferone glucuronide. In addition, the fluorescence is restored by enzymatic hydrolysis with beta glucuronidase. Furthermore, the glucuronide was identified by paper chromatography in two solvent systems. The nonfluorescent umbelliferone glucuronide migrates with an Rf different from that of the nonconjugated fluorescent umbelliferone. Spraying of the paper strip with beta glucuronidase results in hydrolysis of the glucuronide in the paper and appearance of intense fluorescence at the Rf of the glucuronide.

Liver was obtained by aspiration biopsy and homogenates were immediately incubated with 4-methyl umbelliferone and UDPGA. Umbelliferone glucuronide was determined in Units (Figure 5). One

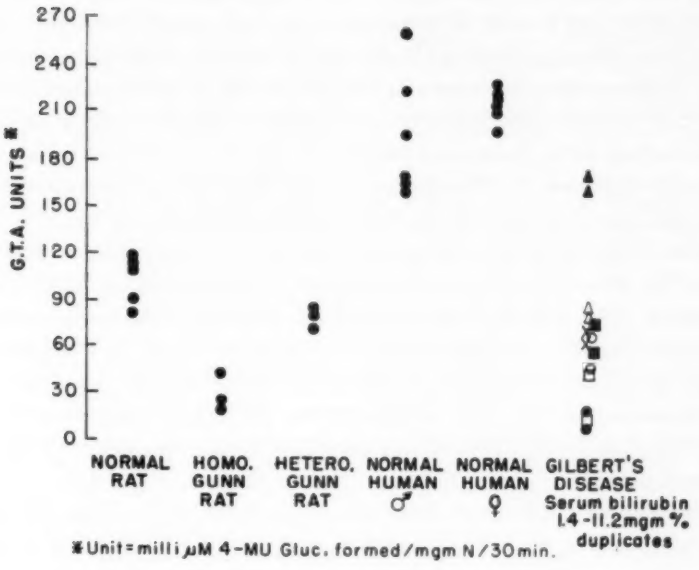

Fig. 5

TABLE IV.—LIVER GLUCURONYL TRANSFERASE ACTIVITY IN PATIENTS WITH NONHEMOLYTIC ACHOLURIC JAUNDICE

Patient	*Serum Unconjugated Bilirubin (mgm. %)*	*Liver Glucuronyl Transferase Activity*
P.H.	13.5	44 UNITS (*)
H.J.	10.5	30
B.H.	8.8	62
N.H.	6.2	84
V.H.	5.5	49
J.A.	5.5	99
S.B.	3.9	115
M.M.	2.8	110
H.K.	2.0	202
J.M.	1.4	156
NORMALS (15)	———	158-267

*UNIT milli μ M 4-M-UMB. GLUC. Formed/mgmN/30min.

Unit equals one milli micromole of 4-methyl umbelliferone glucuronide formed per mgmN of liver in 30 minutes.

The activity of normal human liver as obtained from eleven healthy adult males and females is indicated. Liver glucuronyl transferase activity was virtually absent in the homozygous Gunn rat whereas variable transferase activity was noted in the nonjaundiced heterozygous Gunn rat. One patient with nonhemolytic acholuric jaundice was found to have normal liver glucuronyl transferase activity (indicated by closed triangles in Figure 5). This patient's serum bilirubin concentration was 1.4 mgm. per cent. According to the studies of Alwall and others[21], this degree of bilirubinemia may represent the limit of a normal distribution curve of serum bilirubin concentrations as determined in normal individuals. The data regarding ten of the patients with nonhemolytic acholuric jaundice are presented in more detail in Table IV. The normal range of glucuronyl transferase activity as determined in 15 normal individuals is 158-267 Units. It is apparent that glucuronyl transferase activity is markedly reduced in patients with bilirubinemia over about 5 mgm. per cent.

Because of the extreme sensitivity of the method, the observed difference between the normals and the patients with bilirubinemia of less than about 5 mgm. per cent may not be meaningful. The conjugation mechanism in these latter patients, and attempts to detect nonicteric carriers in the families of these patients, are currently under study. Although one cannot definitively say at this time, the results of *in vitro* assays of liver glucuronyl transferase activity suggest that a spectrum of severity exists in nonhemolytic acholuric jaundice in adults and that the entity may represent varied degrees of expressivity of the same functional defect. At the same time one cannot exclude the possibility that the mechanism responsible for hepatic uptake of bilirubin may play an important role in the very mild degrees of jaundice seen in some patients.

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MEN, MEDICINE, AND CELLS*

Jerome J. Van Gasse**

Any discussion of the role the pharmaceutical industry has played in the evolution of a given class of drugs must begin with the recognition that much of the history involved is quite recent. Thus, though botanicals are as old as man, the American pharmaceutical industry does not date back much further than the Civil War. As for biologicals, they became scientifically important in medicine only a century ago, and some of the most significant developments are less than ten years old.

Interest in biologicals, in fact, has waxed and waned frequently since Parke-Davis obtained biological license Number One from the United States government in 1894. Today, as a result of industry's massive preparation for Salk and influenza vaccine production, interest is again running high.

Both fields, furthermore, are now on the verge of a revolution. There are events lying in the immediate future which may change the nature of biologicals and botanicals production drastically. Just as medical research itself is today concentrating more and more on what happens at the cellular level, so the future of biologicals and botanicals seems to lie in the understanding and control of basic cellular processes.

It is remarkable that a living organism so remote from man as the purple foxglove should produce four chemical compounds with specific action upon highly specific cells of the human body. What use does a purple foxglove have for a drug which regulates the beat of the human heart or for a drug which counters the pituitary antidiuretic hormone? Yet there the drugs are, and there are many in nature like them. On the cellular level, however, the similarities between man and the foxglove are greater than the differences, and the question no longer seems quite so puzzling. We are now beginning to understand that one of the best ways

* Presented, as part of a program, *A History of the Drug House*, at the Thirty-first Annual Meeting of the American Association for the History of Medicine, held at The New York Academy of Medicine and at The Rockefeller Institute, New York, N. Y., May 23, 1958.

**General Manager, Pfizer Laboratories Division, Chas. Pfizer & Co., Inc., Brooklyn, N. Y.

to find a compound with a highly specific action on a living organism is to get another living organism to produce it for you. The medieval German herbalists shared this belief; their motto was, "Gegen den Tod ist kein Kräutlein gewachsen"—"Against death, doth no simple grow", implying that there were surely herbs and simples against every other ailment of man, could one but find them.

With such an inheritance of faith in galenical remedies, it was only natural for the infant pharmaceutical industry to begin with botanicals, and some, such as S. B. Penick & Co., continue to make regular contributions in this field. Chas. Pfizer & Co. started out 109 years ago to manufacture santonin. This drug is probably unknown to most physicians today, although it still has some small currency among veterinarians, but in those days it was a popular vermifuge. It was usually made from the dried, unexpanded flower heads of the Levant wormseed, *Artemesia cina*, which grows mostly in Russian and Chinese Turkestan and in the southern Ural mountains. In those days, harvesting this flower was likely to be attended by some difficulties, owing to continual violent difference of opinion between the local Uzbeks and the Russians.

In our case, the transition from botanicals to fermentation chemicals began almost immediately, although we were not aware of it at the time. It dates from 1862 when we began to produce tartaric acid and cream of tartar, which were made from wine-lees. Eighteen years later we began making citric acid, which was then a botanical, extracted from citrus culls; but by 1923 we had made citric acid a fermentation product, and it still is. One can, however, call a fermentation chemical a botanical without stretching the definition totally out of shape; it is a drug from a plant source. In this sense, then, cannot all antibiotics be considered botanicals? At least, they occupy the middle ground between botanicals and biologicals.

The essence of the change from lemons to fermentation is this: That the crossing has been made from a multi-celled plant source to a single-celled plant source. In another recent instance, that of the steroid drugs, multi-celled and single-celled organisms have been yoked together to attain the desired result. The raw material most, if not all, makers of steroids use is a sapogenin extracted from a Mexican yam. The conversion of this substance into the particular steroid desired is then entrusted to a microorganism. One such steroid, by the way, is hydroxydione or Viadril, an anesthetic, which, as the only intravenous anes-

thetic in use today which can be classified as a botanical, is in the direct line of inheritance from the mandrake root of Egypt and the Orient.

Biological drugs have a shorter history, but the fundamental principle is the same: For a drug with specific action, we again go to a living organism, or to a chain of living organisms. In some cases, the procedure is reminiscent of "the house that Jack built": A pneumococcus is urged to elaborate an antigen, a rabbit is urged to respond with an antibody, and the human body is asked to accept the antibody as if it were its own. An enormously long evolutionary ladder has been climbed in the process, but none of the physical organisms involved seem to care about this—for on the cellular level, all organisms seem to be brothers.

I stress this fundamental unity of principle because there was a time, not too long ago, when there was a widespread tendency to write off biologicals as outmoded. For many years, the biologicals offered the only real hope of preventing and treating infectious disease. The leaders in this field were and are Lederle Laboratories, Eli Lilly, and Parke-Davis and Company, to whom we owe mass production of the vaccines, antisera, toxoids, and other biologicals which made such vast contributions to individual and public health. Yet when the sulfa drugs, penicillin and the broad-spectrum antibiotics came along, the assumption was widely made that biologicals were obsolete.

This was not a logical assumption, for anyone who reflects a moment will realize that the great role of biologicals is now and always has been preventive, and I think most physicians will agree that prophylaxis is the first duty of medicine; therapy, though important, comes second. It is true that there is not much call for pneumonia antisera any longer; penicillin has replaced them. But smallpox vaccine, tetanus toxoid, diphtheria antitoxin all retain their status as medical staples. When smallpox appeared in New York City in March of 1947, it set off the biggest and the fastest mass vaccination campaign in medical history; anyone who still believed after that experience that chemotherapy was going to replace immunology was not looking at what was before his eyes.

In animal health as well as human medicine the continuing importance of biologicals is undiminished. Here these products play a crucial role against hog cholera, Newcastle disease of poultry, and other threats to the farm economy, re-crossing the line from animal to human medicine in the case of rabies.

The Salk vaccine is the most spectacular recent incident in the re-

surgence of immunology, and, more broadly, of interest in the field of living cells as sources of drugs. High though its specific importance is, I think the Salk vaccine has a broad general implication which is even more important: The re-entry of many firms into the field of biologicals, which was triggered by the Salk vaccine, may well represent the tacit abandonment of the hope that we shall ever find a uniformly effective chemotherapeutic agent against the small viruses. Or, to put the matter another way, we shall find some such agent, but we shall ask the human body, or some other living system, to make it for us.

As an example of the resurgence I've mentioned, Pfizer has recently launched a biologicals research program. We have planned a broad series of projects including several with special emphasis on cell culture and viral biologicals. Research will include basic studies, as well as investigations aiming at manufacturing a wide line of human and veterinary vaccines.

In common with some other pharmaceutical firms, we are particularly interested in exploiting the advantages of the tissue culture method of vaccine production, as opposed to older methods. There can now be no doubt that the methods of tissue culture demonstrated in 1948 by Dr. John F. Enders represent a major advance not only for the study of virology itself, but in the whole broad area of industrial biologicals technology. From the manufacturer's point of view, tissue culture allows a cleaner, drier, more stable product; it is cheap; it is fast; and in some cases it can be mated with fermentation techniques, the area where our major experience at Pfizer lies. It is a further instance of the advantages offered in pharmaceutical production by recourse to nature's basic laboratory, the living cell.

Many industrial researchers also share the general dissatisfaction with the traditional calf-lymph method of growing and harvesting vaccinia virus. By today's standards this is a rather crude process, and it is not surprising that it should be—after all, it is better than sixty years old. There seems to be no theoretical reason why tissue culture methods cannot be adapted to this end. Like many other firms, we are also interested in the very difficult problem of growing the measles virus and developing either a killed-virus or an attenuated live-virus vaccine; we are hoping that tissue culture will provide us with a highly versatile tool in this area as well.

From the physiologist's point of view, animal tissue culture raises

many problems in basic research, because the necessary nutritional substrate for the cells must consist in part of plasma, serum, fibrin clots, embryonic extracts, and other substances which are themselves so complex as to becloud nutritional studies of the growing tissue itself. Plant tissue cultures, which can be grown on wholly synthetic substrates, are much easier to study, and much progress has been made in this field.

Recently, a Pfizer phytochemist, Dr. Louis G. Nickell, has opened up a prospect which may well make history for the pharmaceutical industry: The culture of *single* separated plant cells *in vitro*. From parent tissue isolated in 1951, Dr. Nickell and his associates first succeeded in propagating whole populations of separated, individual pole bean plant cells (*Phaseolis vulgaris*) in a liquid medium. These cells remain individuals, and increase in population approximately 15 times in the course of a month. Since publishing this work at the end of 1956, Dr. Nickell has expanded it to include a considerable variety of plants.

This work looks toward the possibility of obtaining botanicals by the methods also used to produce biologicals – in effect, a form of fermentation using cells derived from multicellular organisms – the higher plants – instead of single-celled organisms such as yeasts. Perhaps a new term will be needed to describe drugs made in this way, for the method completely obliterates the traditional division between the two kinds of drugs. For example, it might be possible to grow individual cells of *Rauwolfia* en masse, and obtain reserpine or rescinnamine in this manner, instead of extracting the alkaloids from whole roots imported from India or Latin America. Theoretically, in fact, the same thing might be done to obtain any true botanical.

This is a revolutionary prospect, opening vistas of completely in-plant botanicals production, freed of the vicissitudes of crop-raising and harvesting, such as the weather, economic set-backs such as the Indian ban on the export of Rauwolfia root, and the hazards of field-collecting such as the war between the Uzbeks and the Russians mentioned earlier. This development, for instance, may obviate such searches as that conducted in Africa in the forties, by both industry and the government, for a species of the strophanthus vine which would give a good yield of an aglucone called sarmentogenin, which appeared to be a good starting material for the synthesis of cortisone. This expedition, conducted jointly by the Upjohn Company and S. B. Penick Company, covered

11,000 miles, 12 countries, and six months, and returned without any suitable plants – surely a prime example of the hazards of field collecting. At least, we know now that it is possible to grow plant cells at home under roughly the same conditions that are used for large-scale growth of microorganisms.

What is more, there is no theoretical obstacle to the next logical step, which is the mass growth of individual *animal* cells by fermentation techniques. The practical problems would obviously be enormous, but they may also be well worth the struggle, since animal tissues elaborate many useful drugs–insulin, for example, where Lilly was a pioneer in production–which chemists cannot yet produce by industrially practicable methods. Now, would the result be a biological or a botanical? It seems to me that it would be neither. The result would be drugs produced by living cells, and that is all that biologicals and botanicals are; the traditional division has no reality on the cellular level.

Mass culture of single plant cells is a long-accomplished fact in the field of naturally unicellular organisms such as bacteria, yeasts and molds, but this field has not yet been explored to its ultimate end. One of the gravest medical problems of our time is starvation; and from the industry's point of view, it is also one of the gravest production problems. To some extent we have already called upon the living cell to mass-produce micro-nutrients such as vitamin B_{12} for us. Lately our company has been producing a macro-nutrient, l-lysine, by the same method. Can cell culture also be called upon to supply us with supplemental amounts of whole proteins and fats?

This is being done in some parts of the world, with the cultivation of yeasts as foods. Most of the output goes to livestock, but there is no reason why human beings cannot eat it too; I am told that it even has a pleasant flavor. In recent years much attention has been centered upon the possibility of the mass production of algae for this purpose. The species usually favored is *Chlorella*, because it can be made to vary its protein-to-fat ratio by simple variations in its environment.

Two of our research workers, Dean R. Thacker and Harold Babcock, felt that our experience with the biochemical engineering aspects of handling large unicellular populations could be put to good use in handling *Chlorella*, and in consequence built a pilot plant for the mass culture of this alga. It gave trouble-free operation for almost a year, and gave back better than 14 grams of dry *Chlorella* per square meter

per day. This compares favorably with the results achieved by other workers.

Their conclusions from this test offer little hope that mass algae culture will compete with other foodstuffs in the near future, for even under the most favorable circumstances it could not be produced for under fifty cents per pound. Whole ground soy beans now sell for about six cents a pound. However, there are two possible future developments which might change this picture. First, the current world population explosion may well make even the cheapest foods so scarce as to drive their prices up to where *Chlorella* might be competitive; this is a gloomy prospect, but not an unlikely one. Second, we may yet find some way to mass-produce algae which give far better yields than any we have been able to achieve with the best apparatus ot today. Certainly the problem deserves further exploration.

We have, in truth, come a long way from the blind empiricism of the old herbalists, who had no better theory to guide them than the doctrine of signatures; in fact, we are beginning to leave behind even such modified empiricism as was inherent in the search for antibiotics. We still have much farther to go, however. As we narrow our attention to the living cell, we are reminded that we must not rest content until we know with complete specificity what the chemical bases of disease processes are, and specifically how drugs act to change those processes. That is perhaps the most difficult problem medicine has ever faced, the problem of life itself; but there is no doubt in my mind that we are on our way to finding the answers.

VALENTINE MOTT, 1785-1865
THE ACADEMY'S THIRD PRESIDENT

On NOVEMBER 27, 1865, the members of The New York Academy of Medicine met in the large chapel of the University of the City of New York to hear an address by Dr. Alfred C. Post on "the Life and Character of the late distinguished Fellow and ex-President. . . . Valentine Mott." "A large and appreciating audience of ladies and gentlemen were present." Thus did the Academy honor its illustrious third president who had died six months before, on April 26. No other Fellow of the Academy had won such world-wide acclaim as this great surgeon.

Valentine Mott was born on August 20, 1785, in Glen Cove, Long Island, the son of a physician, Henry Mott. At the age of nineteen he entered the office of his relative, Valentine Seaman, one of the leading New York physicians of the time. Mott received his M.D. degree from

the Medical Department of Columbia in 1806, and in the following year was off to Edinburgh and London for further study. He had been a pupil of the New York surgeon, Wright Post. In London he studied under another great surgeon, Sir Astley Paston Cooper. On his return to New York he taught surgery and embarked upon an extraordinary career as a bold, skilful and pioneer surgeon. He won acclaim throughout the world for the operations he originated. Cooper said of him that he performed more major operations than any surgeon in history up to his time. In the Academy *Bulletin*, 1925, vol. 1, pp. 209-214, F. H. Garrison provides a biographical sketch of Mott which contains, on p. 210, a list of some of these operations. Another biographer claims that he performed about one thousand amputations, 150 operations for stone in the bladder and ligated 40 large arteries. He was the first to tie the innominate artery for subclavian aneurism and to excise the lower jaw for necrosis. He was one of the first to achieve a successful amputation of the hip joint.

Mott was an obvious choice for Academy President. His was one of the three names signed to the public notice, requesting regular practitioners to meet to establish an "Academy of Medicine and Surgery", in December of 1846. His was the first scientific paper, delivered at the meeting of March 3, 1847, on the treatment of ununited fractures. The development of the Academy was of paramount importance to him, and he urged in his valedictory address, February 6, 1850, that the meetings be devoted more to scientific subjects, for he felt that the Academy in its three years of life had not advanced medical science as anticipated. Mott served a second term as President when he was elected in 1857 at the age of seventy-two.

His concern for the Academy is shown in the following exhortation, culled from his valedictory address of 1850:

"Consider, brethren, how dispiriting it is, to sit out a meeting with only thirty or forty members, scattered about the benches in this spacious Hall; how little inducement is there to come again – to *work* for so small an assembly. Think for a moment on the INFLUENCE of these things, and for the Academy's sake – for the sake of *Legitimate Medicine*, assailed by Quackery on every side – for your own sakes – for *mine*, if you will, COME to the meetings of the Academy. Do not, on this pretence, or the other–above all, do not from indolence, abstain

from them. Come, meet, meet, brethren, numerously; and you will make the Academy all that you desire to see it. Let us, on each stated occasion, be enabled to say with the Apostle, 'we are all here,' and all will be well.

"There is nothing that will contribute more to the advancement of the interests of the Academy – nothing that will more conduce to its stability – than punctuality of attendance on the part of all. And when we do meet, let us not talk, *but act*."

GERTRUDE L. ANNAN
Librarian,
The New York Academy of Medicine

A note regarding the illustration–The Academy Library has two valentines made in honor of Valentine Mott by an "artist in penmanship" of Stuyvesant Institute, David Davidson. One, executed in very fine calligraphy, is dated the Ides of February, 1833, and contains the Book of Job. The valentine shown here contains in the tiny script Psalms 67, 103, 104, 107, 108, 113, 117 and 145. The date is November 15, 1855.

Communication From

THE ROYAL SOCIETY OF MEDICINE
Secretary, R. T. Hewitt, O.B.E., M.A.
1 Wimpole Street — London, W. 1, England

23rd April 1959

Dear Dr. Craig,

I am not sure whether you know that the New York Academy of Medicine and this Society agreed in July, 1928, on a form of reciprocity whereby Fellows of this Society visiting New York and bearing letters of introduction would have access to the Academy's House and assistance from its staff whilst in return Members of the Academy visiting London and bearing letters of introduction would have the same privileges here.

The Council of the Society, at a meeting held earlier this week, took note of this arrangement and of the fact that no advantage had been taken of it recently. They feel strongly that the arrangement is one which would be most welcome to Fellows of the Society visiting the United States and they hope that it will be equally welcome to your Members when they are in this country.

I was therefore instructed to write to you to ask you whether you would be agreeable to a continuance of this arrangement, originally proposed to the Society by your International Medical Relations Committee, and to its being made more widely known by our two organizations to their members. Council hope very much that you will be agreeable to this suggestion and want me to assure you that, if it is confirmed, Members of the Academy in London will be given a very warm welcome here.

With kind regards,

Yours sincerely,
R. T. HEWITT, O.B.E., M.A., *Secretary*

Dr. Howard R. Craig,
New York Academy of Medicine,
2 East 103rd Street, New York 29.

Reply

THE NEW YORK ACADEMY OF MEDICINE

April 27, 1959

My dear Mr. Hewitt:

Thank you for your letter of April 23rd. I did not know that there had been any agreement between The Royal Society of Medicine and The New York Academy of Medicine, but I certainly welcome this opportunity for reciprocity. I am most appreciative of your bringing this matter up.

We, of course, will be very happy to do all we can for the Fellows of The Royal Society of Medicine in every possible way. This evidence of reciprocation and hospitality I know will be most welcome and I will circularize this fact amongst our Fellows. We in turn will extend to Fellows of The Royal Society of Medicine a warm welcome on this side of the water.

With kindest personal regards, I am,

Sincerely yours,
HOWARD REID CRAIG, M.D., *Director*

Mr. R. T. Hewitt, O.B.E., M.A.
The Royal Society of Medicine
1 Wimpole Street, London, W.1, England

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THE NEW YORK ACADEMY OF MEDICINE

THIRD ANNUAL POSTGRADUATE WEEK

OCTOBER 5 to 9, 1959

RESEARCH CONTRIBUTIONS TO CLINICAL PRACTICE

PANEL MEETING — 4 P.M.

Monday October 5

PHYSIOLOGY AND THERAPY OF THE GASTRECTOMIZED PATIENT

Chairman: THOMAS P. ALMY, *Cornell University Medical College*

LECTURES — 8:30 P.M.

CONTRIBUTIONS OF MOLECULAR BIOLOGY TO MEDICINE

FRANCIS O. SCHMITT, *Massachusetts Institute of Technology*

THE ESSENTIAL NATURE OF GLYCINE IN THE DYNAMICS OF THE CELL

DAVID RITTENBERG, Ph.D., *College of Physicians and Surgeons, Columbia University*

PANEL MEETING — 4 P.M.

Tuesday October 6

LONG-TERM RESULTS OF SURGERY FOR ACQUIRED CARDIOVASCULAR DISEASE

Chairman: CHARLES E. KOSSMANN, *New York University College of Medciine*

LECTURES — 8:30 P.M.

FACTORS INVOLVED IN CAUSING CONGENITAL MALFORMATIONS

JAMES G. WILSON, *University of Florida College of Medicine*

ADRENOGENITAL SYNDROME: ITS CAUSES, MANIFESTATIONS AND TREATMENT

LAWSON WILKINS, *The Johns Hopkins University School of Medicine*

CONTRIBUTIONS OF PNEUMOCOCCAL GENETICS TO FUNDAMENTAL PROBLEMS IN BIOLOGY

ROBERT AUSTRIAN, *State University of New York, Downstate Medical Center*

PANEL MEETING — 4 P.M.

Wednesday October 7

IATROGENICALLY INDUCED LIVER DISEASE

Chairman: GERALD KLATSKIN, *Yale University School of Medicine*

LECTURES — 8:30 P.M.

CELLULAR EFFECTS OF IONIZING RADIATION

HENRY S. KAPLAN, *Stanford University School of Medicine*

(continued on following page)

LECTURES — 8:30 P.M. (continued)

Wednesday October 7 *(continued)*

NEWER METABOLIC ASPECTS OF TRAUMA
JOHN M. KINNEY, *Harvard Medical School*

ENDOTOXIN, FEVER AND SHOCK
IVAN L. BENNETT, *The Johns Hopkins University School of Medicine*

PANEL MEETING — 4 P.M.

Thursday October 8

FERTILITY AND INFERTILITY
Chairman: JOHN ROCK, *Harvard Medical School*

LECTURES — 8:30 P.M.

GLYCOLYTIC PATHWAYS AND THEIR RELATIONSHIP TO LIPID METABOLISM
MARVIN D. SIPERSTEIN, *The University of Texas, Southwestern Medical School*

THE METABOLISM OF THE SERUM LIPOPROTEINS AND ITS RELATIONSHIP TO ATHEROSCLEROSIS
HOWARD A. EDER, *Albert Einstein College of Medicine*

THE PRESENT STATUS OF THE DIETARY FAT-SERUM LIPID PROBLEM
EDWARD H. AHRENS, *Jr., The Rockefeller Institute*

PANEL MEETING — 4 P.M.

Friday October 9

PROBLEMS IN HYPERSENSITIVITY
Chairman: H. SHERWOOD LAWRENCE, *New York University College of Medicine*

LECTURES — 8:30 P.M.

COPPER METABOLISM AND CERULOPLASMIN
I. HERBERT SCHEINBERG, *Albert Einstein College of Medicine*

IRON METABOLISM AND THE PATHOGENESIS OF IRON DEFICIENCY ANEMIA
CARL V. MOORE, *Washington University School of Medicine*

THE METABOLISM AND THE INTESTINAL TRANSPORT OF CALCIUM
DAVID SCHACHTER, *College of Physicians and Surgeons, Columbia University*

SCIENTIFIC EXHIBIT

The Scientific Exhibit will open officially at 11 a.m. WEDNESDAY, SEPTEMBER 30, 1959 and be open daily 10 a.m. to 10 p.m. through October 9, except Saturday and Sunday, October 3 and 4.

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In the current year the Committee on Medical Education will award *one* Scholarship grant in the amount of $4,500.00 for the *academic year 1960-1961.*

This Scholarship was established by the donor to provide aid for advanced study abroad in a clinical subject in preparation for the practice of medicine or surgery in any of their branches.

As stipulated by the donor of the fund, candidates must be *male* interns or residents in voluntary, municipal or Veterans Administration Hospitals in New York City.

The course of study, the auspices under which it is to be taken and the place or places where the study is to be pursued must receive the approval of the Committee. Applicants are favored who, from their records, show themselves capable of advanced work in clinical medicine. The Committee stipulates that the study be pursued abroad, preferably in Great Britain or in Continental Europe.

Applications for this Scholarship must be submitted by *SEPTEMBER 1, 1959.*

Candidates may secure application forms by addressing:

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The New York Academy of Medicine
2 East 103 Street, New York 29, N.Y.

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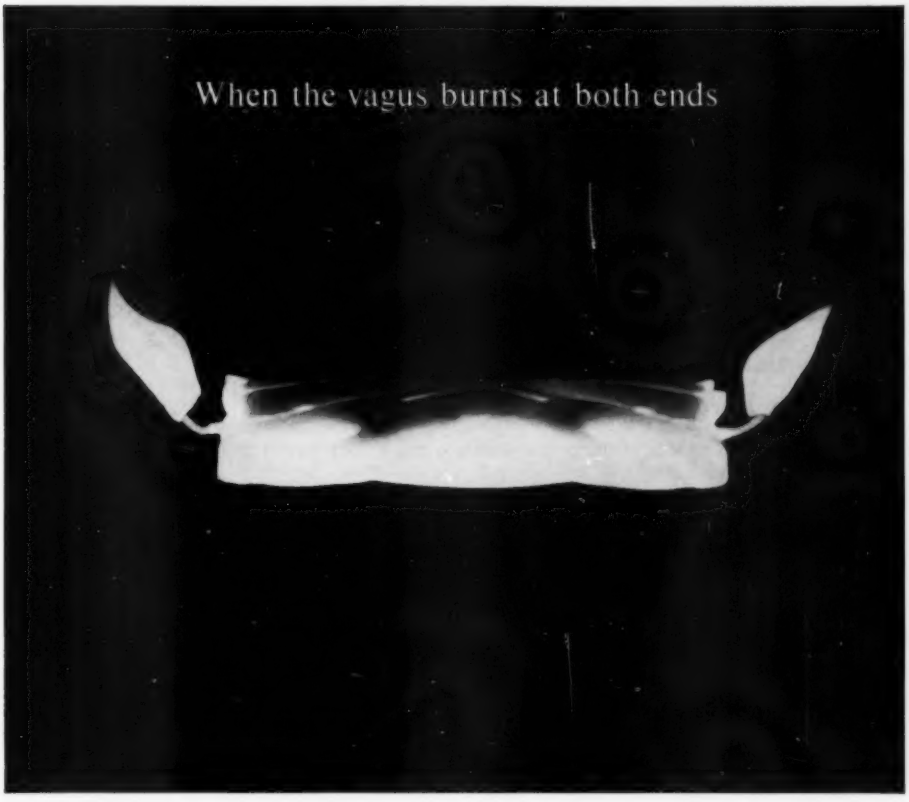
Fellows are reminded that the Library is eager to acquire the publications of all international congresses, their programs, bulletins, proceedings, reports. Since these are often hard to find, it would help a great deal if Fellows attending such congresses would turn over to the Library any such papers as they themselves do not wish to keep. Such thoughtfulness would be deeply appreciated.



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Callaway[3], in a comparison study of 62 patients with various dermatoses treated with ARISTOCORT CREAM and hydrocortisone, concluded that ARISTOCORT CREAM 0.1% is as effective as 1% hydrocortisone in comparable conditions. "In no instance have we seen any evidence of sensitization develop and in no patient has there been any evidence of primary irritation." He describes ARISTOCORT CREAM as "a welcome addition to our dermatological armamentarium."

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References: 1. Blau, S., and Kanof, N. B.: Clinical Report, cited by permission. 2. Orentreich, N.: Clinical Report, cited by permission. 3. Callaway, J. L.: Clinical Report, cited by permission. 4. Robinson, R. C. V.: *Bull. School Med. Univ. Maryland,* 43:54, July, 1958.

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THE NEW YORK ACADEMY OF MEDICINE

Third Annual

POSTGRADUATE WEEK 1959

October 5 — 9, inclusive

RESEARCH CONTRIBUTIONS TO CLINICAL PRACTICE

All inquiries should be addressed to:
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